

CHAPTER

4

# Immunology and Serology

## contents

### Outline 422

- Introduction to Immunology
- The Immune System
- Major Histocompatibility Complex
- Nonspecific Immune Response
- Adaptive Immune Response
- Autoimmune Disease
- Hypersensitivity
- Immune Deficiency
- Hypergammaglobulinemia
- Transplant Immunology
- Tumor Immunology
- Antigen-Antibody Reactions
- Precipitation Reactions
- Agglutination Reactions
- Complement Fixation
- Labeled Reactions
- Cellular Assays
- Streptococcal Serology
- Syphilis Serology
- *Borrelia burgdorferi* Serology
- Rubella Serology
- Epstein-Barr Virus Serology
- Viral Hepatitis Serology
- Human Immunodeficiency Virus Serology

### Review Questions 469

### Answers & Rationales 481

### References 493

## I. INTRODUCTION TO IMMUNOLOGY

### A. Definitions

1. Immunity is the processes that occur to defend the body against foreign organisms or molecules.
2. Immunity includes:
  - a. Inflammation
  - b. Complement activation
  - c. Phagocytosis
  - d. Antibody synthesis
  - e. Effector T lymphocytes

### B. Types of Immunity

1. **Innate** (nonspecific or natural)
  - a. **Born with it**, do not need prior exposure
  - b. The effectiveness of the immune response varies with age.
  - c. **First line of defense**: Designed to keep microorganisms out
    - 1) **Physical barriers**, such as epithelial cells (intact skin), trapping of bacteria in mucus, etc.
    - 2) **Chemicals** secreted by cells and tissues, such as acidic pH of skin surface, complement, interferons, lysozymes, etc.
  - d. **Second line of defense**
    - 1) **Phagocytosis**: The process of a white blood cell (WBC) engulfing bacteria
    - 2) **Inflammation**: Nonspecific response to tissue damage that includes
      - a) Chemical release
      - b) Cellular movement
      - c) Elimination of foreign material
      - d) Tissue repair
    - 3) **Complement system**: Enhances phagocytosis, stimulates inflammatory response, and lyses foreign cells
2. **Adaptive** (specific or acquired)
  - a. Acquired only after a specific challenge is encountered and responds specifically to the challenge
  - b. Two responses
    - 1) **Humoral-mediated immunity (HMI)**
      - a) More important in protection against extracellular pathogens
      - b) Antibody production by plasma cells
    - 2) **Cell-mediated immunity (CMI)**
      - a) More important in protection against intracellular pathogens
      - b) **Natural killer (NK) cells**: Some activity against tumor cells
      - c) **T helper cells**
      - d) **Cytotoxic T lymphocytes (CTLs)**
      - e) **Cytotoxins**

c. **Active immunity**

- 1) **Natural:** The host is exposed to foreign immunogen as a result of infection, and the host's immune cells manufacture specific products to eliminate foreign immunogen.
- 2) **Artificial:** Vaccination; immune system responds to an altered (noninfectious) organism
- 3) Active immunity generally endures for life.

d. **Passive immunity**

- 1) **Natural:** Maternal antibody crosses placenta to protect infant
- 2) **Artificial:** Immune products from another animal injected into the host (e.g., pooled gamma-globulin)
- 3) Passive immunity short term; no memory cells produced

e. **Antigens and immunogens**

- 1) **Immunogen:** A substance capable of inducing an immune response
  - 2) **Antigen:** A substance that specifically interacts with cells or substances of the immune system. Immunogens are also antigens, but not all antigens produce an immune response.
  - 3) **Epitope:** The portion of a molecule (i.e., antigen) that binds to an antibody or T cell receptor
  - 4) **Thymic-dependent immunogens:** Molecules that require T helper cells to stimulate antibody formation
  - 5) **Thymic-independent immunogens:** Molecules that initiate antibody production without stimulating T helper cells
  - 6) Immunogenicity characteristics
    - a) **Foreignness:** Must be recognized by the body as "nonself"
    - b) **Size:** Greater than 10 kilodaltons
    - c) **Chemical composition:** Proteins and carbohydrates are the most immunogenic, whereas lipids and nucleic acids are weakly immunogenic.
    - d) **Complexity:** The more complex a molecule, the more immunogenic it becomes.
    - e) Route of entry into the host also determines immunogenicity.
    - f) Dose of immunogen affects immunogenicity.
    - g) **Degradability:** The immunogen needs to be degraded and presented to cells of the immune system.
  - 7) A **hapten** is a low-molecular-weight molecule that alone is too small to stimulate an immune response but can combine with another molecule to induce a response.
  - 8) **Adjuvant** is a compound that enhances an immune response. It is not immunogenic and cannot induce an antibody response alone.
- f. **Antibody** (immunoglobulin [Ig] or gammaglobulin) is a protein that binds to antigens. There are five classes: **IgG, IgM, IgE, IgA, and IgD**. Antibodies primarily migrate in the beta and gamma regions during protein electrophoresis.

- 1) Antibodies are composed of two heavy polypeptide chains and two light polypeptide chains.
  - a) **Light chains**
    - i) Two types: **kappa** and **lambda**
    - ii) Antibodies have only one type of light chain: kappa or lambda.
  - b) **Heavy chains:** Immunoglobulin classes are defined by a unique heavy chain: IgM—mu ( $\mu$ ), IgG—gamma ( $\gamma$ ), IgA—alpha ( $\alpha$ ), IgD—delta ( $\Delta$ ), IgE—epsilon ( $\epsilon$ )
- 2) Every heavy chain and light chain consists of one **variable domain** and one or more **constant domain**.
- 3) The variable domain defines the specificity of an antibody. This portion of the molecule is referred to as the **fragment of antigen binding (Fab)**.
- 4) The **crystalline fragment (Fc)** of the antibody is located at the carboxy-terminus. It is responsible for the biological activity of the molecule, including activating complement.
- 5) Antibody heterogeneity
  - a) **Isotypes**
    - i) Variations between light and heavy chains
    - ii) Defined by constant regions of all antibodies and kappa and lambda light chains
  - b) **Allotypes**
    - i) Species specific variations in the constant domains of heavy or light chains
    - ii) Different alleles of heavy and light chains
  - c) **Idiotypes**
    - i) Variation in the variable region
    - ii) A single clone of cells produces a single idiootype.
- 6) **J (joining) chain:** Multiple monomers of IgM and IgA are linked by a J chain. One J chain is needed for each IgM or IgA molecule that is linked together.
- 7) **Antibody classes**
  - a) **IgG**
    - i) **Predominant serum antibody**, approximately 75% of immunoglobulins in the blood
    - ii) **Subclasses** include IgG1, IgG2, IgG3, and IgG4.
    - iii) **Only immunoglobulin that crosses the placenta**
    - iv) Produced in **secondary (anamnestic)** antibody response
    - v) IgG1, IgG2, and IgG3 activate the classical complement pathway.

b) **IgM**

- i) **Five monomers** linked together by a J chain and interchain disulfide bonds
- ii) 10% of total serum immunoglobulins
- iii) **First antibody produced against an immunogen**
- iv) Produced in both primary and secondary immune responses
- v) It is the **best activator of the classical pathway of complement**—only one molecule of IgM is required.

c) **IgA**

- i) **Serum and secretory forms:** Serum IgA is a single immunoglobulin molecule, whereas secretory IgA is a dimer held together by a J chain.
- ii) Two **subclasses:** IgA1 and IgA2
- iii) Accounts for 15–20% of total serum antibody
- iv) The functions of serum IgA are antigen clearance and immune regulation.
- v) The function of IgA in mucous membranes is to block attachment of viruses, bacteria, and toxins to host cells.

d) **IgD**

- i) Primarily a cell membrane surface component of B lymphocytes
- ii) Short half-life (2–3 days)

e) **IgE**

- i) **Responsible for allergic (type I hypersensitivity) reactions**
- ii) **The Fc portion binds to receptors on mast cells and basophils.** Once attached to mast cells, IgE binding an allergen triggers degranulation of the cell and release of allergic mediators such as histamine and leukotrienes.
- iii) Elevated IgE concentrations are often found during parasitic infections.

8) **Monoclonal antibodies**

- a) **Definition:** Identical antibodies that are produced from a single clone of plasma cells
- b) Found in individuals with multiple myeloma
- c) Monoclonal antibodies also produced in industry by fusing an antigen-sensitized, splenic B lymphocyte with nonsecreting **myeloma cell**, thus creating an immortal cell line that secretes an antibody of a single idiotype.

9) **Quantification of antibodies**

- a) The purpose is to provide information about the functional immune status of an individual.
- b) IgG, IgM, and IgA are quantified using radial immunodiffusion, nephelometry, or turbidimetry.

## II. THE IMMUNE SYSTEM

### A. Myeloid Cells

1. Responsible for nonspecific response
2. **Monocytes and macrophages**
  - a. In the peripheral blood, this cell is a monocyte; in the tissue, it is a macrophage. Tissue macrophages include alveolar macrophage, Küppfer cells (liver), and astrocytes and microglia cells (nervous system).
  - b. Functions
    - 1) **Phagocytosis of invaders**
    - 2) **Present immunogens to T helper cells**, the first step in an immune response
    - 3) Release cytokines (monokines) that affect other cells' activities
  - c. Macrophages have major histocompatibility complex (MHC) class II, complement, and antibody Fc receptors on their surface.
3. **Granulocytes**
  - a. **Neutrophils** (polymorphonuclear cells or PMNs)
    - 1) 60–70% of WBCs in circulation
    - 2) Function: Phagocytosis and contributes to inflammatory response
  - b. **Eosinophils**
    - 1) 1–3% of circulating WBCs
    - 2) Mediate IgE allergic response
  - c. **Basophils**
    - 1) 0–1.0% of circulating WBCs
    - 2) Has receptors for IgE and granules responsible for allergic reactions
4. **Lymphocytes**
  - a. 20–40% of circulating WBCs
  - b. **B lymphocytes (or B cells)**
    - 1) 20% of circulating lymphocytes
    - 2) Express surface molecules such as CD (cluster of differentiation) 19 and CD20.
    - 3) After birth, B cells mature in the bone marrow.
    - 4) B cells differentiate into either a plasma cell, whose role is to produce antibody, or a memory B cell.
  - c. **T lymphocytes (or T cells)**
    - 1) 80% of circulating lymphocytes
    - 2) Express surface molecules such as CD2 and CD3
    - 3) **Functions**
      - a. CTLs lyse host cells infected with viruses and tumor cells and also produce lymphokines.
      - b. T cells stimulate (T helper cells) or suppress (T suppressor cells) other cells.

- 4) **T cell maturation**
  - a) Pre-T cells begin in bone marrow and fetal liver.
  - b) T cells go to the thymus to mature.
- 5) **NK cells** are slightly larger than T or B cells and have cytoplasmic granules.
5. Other cells that assist in the immune response
  - a. **Dendritic cells** present antigen to T cells.
  - b. **Langerhans cells:** Dendritic cell found in the dermis and squamous epithelia
  - c. **Mast cell:** Granulocyte resembling basophil that contains many chemicals that affect the immune response

## B. Cytokines

1. Soluble protein molecules secreted by one cell type that affect other cells, turn on genes in target cells (Table 4-1 ■)

TABLE 4-1 IMPORTANT CYTOKINES

Cytokine	Cellular Source	Primary Target
IL-1	Macrophages, B cells, fibroblasts, etc.	T cells, B cells, macrophages, endothelium, tissue cells
IL-2	T cells	T cells
IL-3	T cells	Stem cells
IL-4	T cells	B cells, T cells
IL-5	T cells	B cells
IL-6	T cells, B cells, fibroblasts, macrophages	B cells, hepatocytes
IL-7	Bone marrow, stromal cells	Pre-B cells, T cells
IL-8	Monocytes	Fibroblasts
IL-9	T cells	T cells, mast cells
IL-10	T cells	TH 1 cells
TNF	Macrophages, mast cells, lymphocytes	Macrophages, granulocytes, tissue cells
IFN- $\alpha$	Leukocytes, epithelia, fibroblasts	Tissue cells
IFN- $\beta$	Fibroblasts, epithelia	Tissue cells, leukocytes
IFN- $\gamma$	T cells, NK cells, epithelia, fibroblasts	Leukocytes, tissue cells, TH 2 cells

## 2. Interferons

- a. Interferon-alpha ( $\text{INF-}\alpha$ ) and  $\text{INF-}\beta$  are antiviral proteins that inhibit viral replication and activate NK cells. They are produced by viral-infected cells.
- b.  $\text{INF-}\gamma$  has antiviral effects, activates macrophages and NK cells, and stimulates B cells to produce antibodies. It is produced by TH 1 cells.

## 3. Tissue necrosis factors

- a. Tumor necrosis factor-alpha ( $\text{TNF}\alpha$ ): Produced by macrophages, lymphocytes, and NK cells when encountering bacteria, viruses, tumor cells, toxins, and complement protein C5a
- b.  $\text{TNF}\beta$ : Produced by CD4 and CD8 positive cells after exposure to a specific antigen

## 4. Interleukins

- a. **Interleukin 1** ( $\text{IL-1}$ ) is produced by macrophages, B cells, and other cell types.  $\text{IL-1}$  activates T helper cells, increases number of B cells, activates vascular endothelium, causes fever and acute-phase protein synthesis, and induces T cells to produce lymphokines.
- b. **IL-2** is produced by T helper cells.  $\text{IL-2}$  causes proliferation of activated T and B cells.
- c. **IL-3** is produced by activated T cells.  $\text{IL-3}$  increases the number of mast cells in skin, spleen, and liver.
- d. **IL-4** is produced by activated T cells.  $\text{IL-4}$  induces proliferation of T cells and class switching from IgM to IgG1 and IgE.
- e. Several other interleukins are known.

## C. Organs and Tissues of the Immune Cells

1. Primary lymph tissues of adults
  - a. **Bone marrow:** Pre-B lymphocytes develop into mature B cells.
  - b. **Thymus:** Pre-T lymphocytes develop into mature T cells.
2. Secondary lymphoid organs
  - a. **Lymph nodes:** B cells migrate to the cortex and T cells to the paracortex.
    - 1) **Primary follicle:** Many small B cells
    - 2) **Secondary follicle:** After stimulation, primary follicle becomes a secondary follicle. The germinal center has small and large lymphocytes, blast cells, macrophages, and dendritic cells. The medulla contains plasma cells and large lymphocytes.
  - b. **Spleen**
    - 1) Purpose: Filter blood
    - 2) Contains both T and B cells
  - c. **Mucosal-associated lymphoid tissue (MALT)**
    - 1) Found in submucosa in gastrointestinal tract, respiratory tract, and urogenital tract



- 2) These surfaces interact with the environment and can begin the immune response early.
- 3) **Peyer's patch:** Specialized MALT found in the lower ileum

### III. MAJOR HISTOCOMPATIBILITY COMPLEX

#### A. Human Leukocyte Antigens

1. Human leukocyte antigens (HLAs) are **cell surface markers** that allow immune cells to distinguish “self” from “nonself.”
2. These antigens were first described on white blood cells (leukocytes) and are coded for by genes in the **MHC** located on chromosome six.

#### B. Three Classes of MHC Products

1. **Class I loci:** HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, and HLA-J
  - a. Molecules found on nearly every nucleated cell surface
  - b. Antigen-presenting cells with MHC I molecules present antigens to CTLs.
2. **Class II:** 13 loci, including HLA-DM, HLA-DO, HLA-DP, HLA-DQ, and HLA-DR
  - a. Molecules located on the surface of monocytes, macrophages, B cells, activated T cells, dendritic cells, Langerhans' cells, and some epithelial cells
  - b. Antigen-presenting cells with MHC II molecules present antigens to T helper cells.
3. **Class III** products: Complement proteins,  $\text{TNF}\alpha$  and  $\beta$ , and other proteins (e.g., heat shock protein) not associated with cell membrane surfaces

#### C. Nomenclature

1. HLA antigens are named according to the product expressed by the gene locus (capital letter) and the allele (number).
2. For example, HLA-A2; A is the locus and 2 is the allele.

#### D. Inheritance of HLA

1. **Haplotype:** Combination of inherited HLA alleles
2. Two haplotypes (one from each parent) are a genotype.
3. Because of the large number of alleles in the MHC, a person's HLA type is almost as unique as a fingerprint.

#### E. Clinical Significance

1. **Transplantation:** Transplants last longer if the HLA antigens from the recipient and the donor are closely matched.
2. **Platelet transfusion:** Although poor response to platelet transfusion is multifactorial, antibodies to class I HLA antigens are the primary cause of immune-mediated platelet transfusion refractoriness.

3. **Paternity testing:** HLA loci are polymorphic and recombination is rare. HLA inheritance patterns can exclude fathers with approximately 99% accuracy.
4. **Diseases:** Not all individuals who have a particular HLA antigen have a disease, but many individuals with certain diseases express a particular HLA antigen. For example, HLA B-27 is associated with ankylosing spondylitis.

#### IV. NONSPECIFIC IMMUNE RESPONSE

##### A. Nonspecific Immune Response: Cellular Mechanisms

1. Barrier, first line of defense: Skin and mucous membranes
2. **Polymorphonuclear neutrophils**
  - a. Involved in nonspecific response by attachment to damaged epithelium, migration into tissues, chemotaxis, phagocytosis and digestion of target cells, increased metabolism, and degranulation
  - b. Polymorphonuclear neutrophil defects
    - 1) **Chronic granulomatous disease:** Defect in oxidative pathway (respiratory pathway) phagocytes use to create hydrogen peroxide, which is used to kill bacteria
    - 2) **Myeloperoxidase (MPO) deficiency:** Impaired production of toxic oxygen molecules (decrease respiratory burst) used by phagocytes to kill ingested bacteria
3. **Eosinophils**
  - a. Granules contain acid phosphatase, peroxidase, histamines, and several other types of molecules.
  - b. **Hypothesized functions**
    - 1) Clearing immune complexes
    - 2) Limiting inflammatory reactions
    - 3) Protein in granules toxic to parasites
4. **Mediator cells**
  - a. Mast cells, basophils, and platelets release substances that mediate immune reactions.
  - b. The mediators produce increased vascular permeability, smooth muscle contraction, chemotaxins for phagocytes, and increased inflammatory response.
  - c. **Mast cells** can degranulate when membrane-bound IgE binds an allergen or by nonimmunologic mechanisms such as surgical incisions, heat, and skin or mucous membrane infections.
  - d. **Basophil function** is to amplify the reactions that start with the mast cell at the site of entry of the antigen. Their granules contain mediators (e.g., histamine and heparin) that play a role in anaphylactic reactions.
5. The mononuclear phagocyte system includes alveolar macrophages, splenic macrophages, Kupffer cells of the liver, etc.

## B. Inflammation

1. Sequenced events following tissue damage that protect the host from foreign invaders and attempt to minimize tissue damage
2. **Increased vascular permeability**
  - a. Upon injury, capillaries, arterioles, and venules are dilated to **increase blood flow** to the site of the injury.
  - b. Because of increased vascular permeability, fluid moves from the circulation to the space around the injury, bringing fibrinogen and PMNs to the injury site.
3. **Migration of neutrophils**
  - a. After the injury, **chemotaxins** and **endothelial activating factors** are released.
  - b. PMNs adhere to activated **endothelial cells**.
  - c. PMNs move between the endothelial cells to the site of tissue damage by a process called **diapedesis**.
  - d. Chemicals are released and more PMNs are released from the storage pool, and the injury site is flooded with PMNs.
4. **Migration of mononuclear cells**
  - a. The macrophages release IL-1, which attracts monocytes, macrophages, and lymphocytes to the injury site.
  - b. About 4 hours after the injury, mononuclear cells migrate to the site of damage.
5. Cellular proliferation and repair: Fibroblasts help repair the damage and return the injury site to normal.

## C. Chemical Mechanisms of the Nonspecific Immune Response

1. **Complement system:** Collection of serum proteins involved in lysis of cell membranes, mediation of inflammation, enhancement of phagocytosis, and metabolism of immune complexes
  - a. **Components are synthesized in the liver**, except C1, which is synthesized in the epithelial cells of the intestine.
  - b. **Approximately 20 proteins involved in three separate pathways of activation**
  - c. Five proteins unique to classical pathway: C1q, C1r, C1s, C4, and C2
  - d. Three proteins unique to alternative pathway: factor B, factor D, and properdin
  - e. Six proteins common to both pathways: C3, C5, C6, C7, C8, and C9
  - f. Activation of complement
    - 1) **Classical pathway:** Immune (antibody-antigen) complexes, require one IgM or two IgG molecules
    - 2) **Alternative pathway:** Antibody-independent, microbial components such as lipopolysaccharide, polysaccharide, teichoic acid, and peptidoglycan

- 3) **Lectin pathway:** Binding of mannose-binding lectin to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms
- g. Outcome of complement activation
  - 1) **Anaphylatoxins:** C4a, C3a, and C5a cause basophils and mast cells to release histamine and also cause smooth muscle contraction and increased vascular permeability.
  - 2) **Immune adherence:** C3b adheres to immune complexes and surfaces of substances to facilitate clearing of these molecules.
  - 3) **Opsonization:** If C3b is attached to a cell, phagocytosis is enhanced.
  - 4) **Chemotaxis:** C5a is an anaphylatoxin and induces the migration of neutrophils and monocytes to the site.
  - 5) Cell lysis through the formation of the **membrane attack complex (MAC)**, components C5 through C9
- h. **Control mechanisms**
  - 1) **C1 inhibitor (C1INH)** combines with C1r and C1s to block C1 activities. A deficiency in C1INH results in the syndrome **hereditary angioedema**, an autosomal dominant disease. The disease is characterized by unregulated classical pathway activation, resulting in vascular permeability and swollen mucous membranes in airways, which can become blocked.
  - 2) **Anaphylatoxin inactivator:** This compound removes a single amino acid from C4a, C3a, and C5a, rendering them useless as anaphylatoxins.
  - 3) **MAC inhibitors:** MAC is not formed because S protein binds to C5b-7 complex.
  - 4) **Complement receptor type I (CRI or CD35):** CRI binds C3b and C4b and inhibits the amplification loop.
- i. **Complement deficiencies**
  - 1) Individuals can have **altered genes**, resulting in complement protein deficiencies (Table 4-2■).

TABLE 4-2 SELECTED COMPLEMENT DEFICIENCIES

Complement Deficiency	Significance
C3	Increase risk for overwhelming infections
C4 and C3	Indicate consumption with classical pathway activation
C1 (q, r, s), C4, and C2	Indicate collagen disease
C5, C6, and C7	Increased risk for <i>Neisseria meningitidis</i> infection

- 2) **Complement can be consumed** in infections and collagen vascular diseases.
  - 3) **C3 and C4 are measured to indicate consumption and follow disease states.**
  - 4) Total functional complement assay (**CH50**) is used to measure the activity of the classical pathway.
2. **Acute-phase reactants:** When injured, the body produces acute-phase reactants (proteins).
- a. **C-reactive protein (CRP)** concentration increases several hundred times after injury. CRP can activate the classical pathway of complement and can also bind to NK cells and monocytes, stimulating them to target tumor cells. CRP levels may also be increased during coronary heart disease.
  - b. **Haptoglobin** removes free hemoglobin from circulation.
  - c. **Fibrinogen** is found in increased quantities at the site of an injury; it is converted to fibrin to heal the injury.
  - d.  **$\alpha_1$ -Antitrypsin** is a family of serine protease inhibitors synthesized in the liver. Deficiency causes premature loss of elasticity in the lung and liver damage.
  - e. **Ceruloplasmin** is the principal copper-transportation protein. It is vital in aerobic energy production, collagen formation, and protection against superoxide ions. Deficiency is called Wilson disease.
  - f.  **$\alpha_2$ -Macroglobulin** is a protease inhibitor.  $\alpha_2$ -Macroglobulin and protease complexes are phagocytized by macrophages and fibroblasts.

## V. ADAPTIVE IMMUNE RESPONSE

### A. Antigen Recognition

1. **Antigen-presenting cells**
  - a. **Monocytes/macrophages:** Phagocytic cells that process antigen and express it on the cell surface associated with MHC I or II molecules
  - b. **Dendritic cells:** Phagocytic cells that process antigen and express it on the cell surface associated with MHC I or II molecules
  - c. **B cells:** Nonphagocytic cells that attach to antigens in their native form, process antigens, and express them on their surface associated with MHC II molecules
2. **Antigen receptors**
  - a. **B cell**
    - 1) The B cell antigen receptor is **monomeric IgM or IgD**.
    - 2) The B cell surface receptors have two identical antigen-binding pockets—the Fab portion of an antibody monomer.
  - b. **T cells**
    - 1) The **T cell receptor** consists of two nonidentical peptides and CD3.
    - 2) T cells recognize antigens that were processed by other cells.

- 3) T helper (TH) cells have **CD4** on their surface that interacts with MHC II on the antigen presenting cell.
- 4) **TH cell activation**
  - a) Occurs when TH cells recognize an antigen
  - b) Requires direct cell contact and cytokines such as IL-1 and IL-2
- 5) CTLs have **CD8** on their surface, which interacts with MHC I on the antigen-presenting cell.

## B. Cell-Mediated Immunity

1. **Mediated by TH 1 cells**, a subset of T helper cells, that secrete cytokines that activate other cells involved in the response
2. **Monocytes and macrophages** are stimulated by cytokines from TH 1 cells, inflammatory reaction cells that are activated by cytokines.
3. **CTLs** are activated by cytokines from TH 1 cells and then destroy targets by cell-to-cell contact. The main function of CTLs is to destroy virus infected cells.
4. **NK cells** kill target cells without being previously sensitized. NK cell activities are governed by cytokines.

## C. Humoral-Mediated Immunity

1. **B cell activation** begins when antigen binds to antibody on B cell surface and the antigen is internalized and linked to an MHC II molecule on the cell's surface.
2. **T and B cell interactions**
  - a. B cell processes and presents the antigen, stimulating the TH 2 cell to produce cytokines.
  - b. The **cytokines** stimulate the B cell to divide and differentiate into a memory B cell or a plasma cell that will synthesize antibody.
3. **Antibody diversity**: Antibodies can be produced that recognize an unlimited number of antigens, but there are a limited number of B cells. Antibody diversity is due to recombination events that occur during B cell maturation. **Plasma cells** produce antibodies with the same specificity of the antibodies that were on the surface of the B cell that the plasma cell was derived from.
4. **Antibody production**
  - a. Primary and secondary antibody responses
    - 1) **Primary antibody response**: Produced when host first encounters antigen
      - a) During the latent phase, no antibody is produced for about 5–7 days. During this time, the host is producing plasma cells that will secrete antibodies.
      - b) **IgM** is the first antibody produced.
      - c) Antibody production starts slowly, peaks, levels off, then declines.

- 2) **Secondary (anamnestic) response:** Produced after the host has previously been exposed to an antigen
  - a) Short latent phase (3–5 days)
  - b) Higher antibody concentration
  - c) IgG produced due to class switching
  - d) IgG antibodies persist longer in circulation than IgM.
- b. **Antibody-dependent cell-mediated cytotoxicity (ADCC):** Cytolytic effector cells (e.g., NK cells and PMNs) can lyse antibody coated target cells if there is direct contact.

## VI. AUTOIMMUNE DISEASE

### A. Definition

1. An autoimmune disease occurs when an individual produces antibodies or a T cell response to his/her own antigens.
2. There is a loss of self-tolerance.

### B. Autoimmune Mechanisms

1. Antibody-cell surface component interaction
2. Formation of autoantigen-autoantibody complexes
3. Sensitization of T cells
4. Genetic factors play a role in the development of autoimmune diseases. The presence of certain HLA types has been correlated with specific diseases (Table 4-3■).

### C. Autoimmune Theories

1. **Forbidden-clone theory:** Burnet postulated that when an error in self-recognition occurs during fetal life and lymphocytes against an autoantigen are not destroyed, then autoantibodies are produced.
2. **Clonal anergy:** Clones developed during fetal life are not stimulated by low doses of antigens. The ability to produce antibodies against higher doses of antigens is still present.

TABLE 4-3 HLA TYPE AND ASSOCIATED DISEASES

HLA Type	Associated Diseases
HLA-B8	Graves disease and type 1 diabetes
HLA-DR2	SLE, multiple sclerosis, Hashimoto disease, and myasthenia gravis
HLA-DR3	Sjögren syndrome, myasthenia gravis, SLE, Graves disease, and type 1 diabetes
HLA-DR4	Rheumatoid arthritis, type 1 diabetes, and pemphigus vulgaris



3. **Sequestered-antigen theory:** Some antigens are hidden from the immune system during fetal development. When the tissue is damaged, the “hidden cells” are exposed to the immune system and antibodies are produced against these cells.
4. **Immunologic deficiency theory:** Suppressor T cells control antibody production by B cells. If suppressor T cells exhibit decreased activity, then antibodies against autoantigens are produced.
5. **Molecular mimicry:** An individual can make antibodies or reactive T cells to an infectious agent that cross react with self antigens.
6. **Polyclonal B cell activation:** A number of bacteria and viruses are known to nonspecifically stimulate B cells. If these B cells have activity against self antigens, an autoimmune disease can result.

#### D. Diagnostic Tests for Non-Organ-Specific Autoimmune Diseases

1. **Antinuclear antibodies (ANAs)**
  - a. Associated with **systemic lupus erythematosus (SLE)**, mixed connective tissue disease (MCTD), and rheumatoid arthritis (RA)
  - b. Techniques used to detect ANA: Agglutination, indirect immunofluorescence, and enzyme immunoassay
  - c. Interpretation of indirect immunofluorescence results
    - 1) Diffuse or **homogeneous:** Evenly stains the nuclei and is associated with anti-DNA antibody and histones
    - 2) **Peripheral:** Stains the edge of the nuclei and is associated with anti-DNA antibody and anti-lamins (proteins found in the nuclear membrane) antibody
    - 3) **Speckled:** Numerous evenly distributed stained speckles within the nuclei associated with antibodies to extractable nuclear antigens—nuclear ribonucleoprotein (RNP) and anti-Smith (Sm)
    - 4) **Nucleolar:** Stains two or three large fluorescent areas within the nucleus and is associated with anti-RNP antibody
    - 5) **Centromere:** Stains as a discrete speckled pattern due to anti-centromere antibody
  - d. **Autoantibodies and disease associations** (Table 4-4■)
2. **Rheumatoid factor**
  - a. Rheumatoid factor (RF) is an anti-antibody, **typically IgM**, that binds to the Fc portion of abnormal IgG.
  - b. RF is usually detected by **latex agglutination**. Patient serum is mixed with IgG-coated latex particles. Agglutination indicates the presence of RF.
  - c. **Approximately 75% of patients with rheumatoid arthritis are positive for RF.** However, patients with chronic infections may also have RF.
  - d. Also noted in chronic hepatitis, SLE, and syphilis
3. **Cryoglobulins**
  - a. Proteins that reversibly precipitate at 4°C



TABLE 4-4 AUTOANTIBODIES AND ASSOCIATED DISEASES

Autoantibody	Disease Associations
Centromere	CREST (calcinosis, Raynaud syndrome, esophageal hypomotility sclerodactyly, and telangectasia) syndrome
dsDNA	Found in SLE and low titers found in rheumatoid arthritis and Sjögren syndrome
Histone	Drug-induced SLE
Nuclear RNP	SLE and mixed connective tissue disease
Scl-70	Scleroderma (systemic sclerosis)
Sjögren syndrome A (SSA [Ro])	Sjögren syndrome and SLE
Sjögren syndrome B (SSB [LA])	Sjögren syndrome and SLE
Sm	Diagnostic for SLE (high specificity) if present but low sensitivity

- b. Associated with autoimmune diseases such as vasculitis, glomerulonephritis, SLE, RA, and Sjögren syndrome

## E. Non-Organ-Specific Autoimmune Diseases

### 1. Systemic lupus erythematosus

- a. Chronic, noninfectious inflammatory disease involving many organs
- b. Disease is **more likely to occur in women than men** and in blacks than whites.
- c. **Tissue injury is caused by autoantibodies and immune complexes deposited in the tissues.** Depressed suppressor T cell function allows production of antibodies against “self.”
- d. **Severity of the disease varies.** Symptoms include fever, weight loss, malaise, weakness, arthritis, skin lesions, photosensitivity, butterfly rash (rash across the cheeks and bridge of the nose), renal disease, pericarditis, seizures, ocular changes, pancreatitis, and small-vessel vasculitis.

### 2. Rheumatoid arthritis

- a. Chronic, noninfectious, systemic inflammatory disease that primarily affects the joints
- b. Women are affected 2–3 times more often than men.
- c. The disease is **due to production of IgG or IgM antibodies** against IgG in the synovium. Immune complexes form, which activate complement.

The inflammatory response proceeds and damages the synovium. Immune complexes attract neutrophils and macrophages to the joint that degranulate and contribute to tissue destruction.

- d. **Symptoms** are highly variable and include fatigue, weight loss, weakness, mild fever, anorexia, morning stiffness, joint pain (that improves during the day), vasculitis, and rheumatoid nodules.
  - e. **Laboratory findings** include elevated erythrocytic sedimentation rate (ESR), elevated CRP, positive RF, cryoglobulins, and sometimes ANAs. Synovial fluid is cloudy, with a WBC count between 5000 and 20,000/ $\mu\text{L}$ , elevated protein, poor mucin clot development, decreased complement, and positive RF.
3. **Sjögren syndrome**
    - a. An inflammation of the salivary and lacrimal glands causing dryness of the mouth and eyes
    - b. **Laboratory findings** include polyclonal hypergammaglobulinemia; autoantibodies against the salivary glands; and positive RF, ANA (speckled or diffuse pattern), anti-SSA, and anti-SSB.
  4. **Autoimmune hemolytic anemia**
    - a. Increased rate of red blood cell (RBC) destruction
    - b. Results in a normocytic, normochromic anemia
    - c. Autoantibody is directed against RBC antigens.
    - d. **Laboratory findings** include positive direct antiglobulin test and sometimes cold agglutinins.

## F. Organ-Specific Autoimmune Disease

1. Organ-specific autoimmune disease: Immunologic reactions take place in only one organ.
2. Autoimmune thyroiditis
  - a. **Hashimoto disease**
    - 1) Humoral and cellular immunity are activated and destruction of normal thyroid tissue leads to hypothyroidism, loss of thyroid function, and low levels of thyroid hormones in the blood.
    - 2) **Antithyroid antibodies** detected include antithyroglobulin, antithyroid peroxidase (microsomal antigen), and second colloid antigen (CA-2).
  - b. **Graves disease**
    - 1) The disease is characterized by hyperplasia and diffuse goiter caused by an autoantibody reacting with thyroid receptor on cells that overstimulates the thyroid gland. The autoantibody mimics the activity of thyroid-stimulating hormone (TSH).
    - 2) **Thyrotoxicosis results from overstimulation**; both free and total  $T_3$  and  $T_4$  are elevated, and TSH is decreased.
    - 3) Common findings: Exophthalmos (bulging eyes) and infiltrative dermopathy

### 3. **Myasthenia gravis**

- a. Neuromuscular disease in which the nerve muscles do not function normally
- b. Most patients exhibit **antibodies to acetylcholine receptors**. These autoantibodies block nerve impulses and can initiate damage to neurons.

### 4. **Multiple sclerosis (MS)**

- a. Considered a chronic progressive inflammatory disease with demyelination of the nerves
- b. Studies suggest that certain viruses, in particular Epstein-Barr virus and human herpes virus 6, are associated with MS.
- c. Active lesions (**plaques**) contain CTLs, T helper cells, and macrophages.
- d. Most patients with MS have **increased IgG concentrations in the cerebrospinal fluid (CSF)**.
- e. The IgG index differentiates true increases due to production rather than increases in permeability of the blood-brain barrier.

$$\text{IgG index} = \frac{\text{IgG}_{\text{CSF}}/\text{albumin}_{\text{CSF}}}{\text{IgG}_{\text{serum}}/\text{albumin}_{\text{serum}}}$$

- f. Reference range for IgG index is 0.0–0.77.
- g. **Oligoclonal bands in CSF** on high-resolution electrophoresis are also indicative of MS, but patients with other conditions (SLE, viral meningitis, neurosyphilis, etc.) can have oligoclonal bands in the CSF.

### 5. **Type 1 diabetes**

- a. **Islet cell destruction in the pancreas** results in insulin-dependent or type 1 diabetes mellitus.
- b. **Autoantibodies and CTLs** reactive against pancreatic beta cells produce marked atrophy and fibrosis of the islet cells. This, in turn, causes insulin deficiency.
- c. Viruses can trigger autoantibody production by **molecular mimicry**. After outbreaks of mumps, measles, rubella, Coxsackie B virus, and infectious mononucleosis, new cases of type 1 diabetes appear in communities.
- d. HLA-DQ1.2 and HLA-DR2 decrease the risk of developing diabetes.

## VII. HYPERSENSITIVITY

### A. **Definitions**

1. **Hypersensitivity reaction:** Overreactive immune response to innocuous substances on reexposure that can result in tissue damage
2. Involve humoral- and cell-mediated responses
  - a. Types I through III are humoral mediated and immediate.
  - b. Type IV is cell mediated and delayed.
3. **Allergen:** Molecule that triggers a hypersensitivity reaction

## B. Type I Hypersensitivity Reaction

1. Type I hypersensitivity (anaphylactic) reaction is classified as an **immediate hypersensitivity reaction** because it occurs within minutes after reexposure to an allergen. After the first (primary) exposure, basophils and **mast cells are sensitized with IgE**. Upon second exposure, IgE binds to a specific allergen and chemical mediators are released from those cells (degranulation), which causes allergic symptoms.
2. **Allergens and disease**
  - a. The **magnitude of the allergic response** depends upon where the allergen enters the body. Individuals who exhibit symptoms are genetically predisposed to produce increased amounts of IgE to that allergen.
  - b. Individuals can be **exposed to allergens** through the upper respiratory tract, absorption from the intestinal tract, and direct skin contact.
  - c. **Allergic reactions occur in tissues with many mast cells:** Skin, nasal membranes, tongue, lungs, and gastrointestinal tract.
  - d. Allergens contacting the nasal mucosa cause runny nose, itching eyes and nose, sneezing, and nasal congestion. Eosinophil levels in blood stream and nasal secretions may be elevated, and IgE may be normal or elevated.
  - e. **Allergens contacting the bronchus cause asthma.** Serum IgE levels are usually increased.
  - f. Although food allergies are common, they are the least common form of type I hypersensitivity reactions. Symptoms include nausea, vomiting, cramps, abdominal pain, and diarrhea within 2 hours of ingesting the allergen.
  - g. **Anaphylaxis** is the systemic form of type I hypersensitivity. It can be life threatening, causing shock or edema of the upper respiratory tract. Substances that can trigger this condition include peanuts; seafood; egg albumin; honeybee, wasp, or hornet stings; vaccines; penicillins; or sulfonamides.
3. **Mediators of symptoms**
  - a. **Histamine**
    - 1) Causes contraction of bronchioles and smooth muscle of blood vessels
    - 2) Increases capillary permeability
    - 3) Increases mucus secretion in the airway
  - b. **Prostaglandins** cause vasodilation and increased vascular permeability.
  - c. **Leukotrienes** cause erythema and wheal formation. They have 30–1000 times the ability of histamine to cause bronchospasms and also stimulate mucus secretion in the airways.
4. **Laboratory evaluation of allergies**
  - a. **Total serum IgE levels**
    - 1) Methods used include competitive radioimmunosorbent test (RIST), noncompetitive RIST, double-antibody radioimmunoassay (RIA), and sandwich enzyme-linked immunosorbent assay (ELISA). Despite the name, radioimmunosorbent, radioisotope labels are generally no longer used. They have been replaced with enzyme-labeled assays.

- 2) In the **competitive assay**, a quantity of labeled anti-IgE is mixed with serum containing IgE. The IgE in the patient sample competes with the labeled IgE for binding to anti-IgE attached to a solid surface (e.g., microtiter plate). The more IgE present in the sample, the lower the signal produced by the label.
  - b. **Allergen-specific IgE:** The radioallergosorbent test (RAST) is used to detect IgE against specific allergens. The test can be performed as a competitive or noncompetitive assay.
5. **Treatment**
- a. Allergen avoidance and drug therapy
  - b. Patients can undergo **immunotherapy (hyposensitization)**, commonly referred to as “allergy shots.” Individuals receive injections of gradually increasing concentrations of the allergen to which they are allergic. Eventually, a state of **tolerance** to the allergen may develop.

### C. Type II Hypersensitivity

1. Type II hypersensitivity (cytotoxic) reaction is due to **IgG or IgM antibodies directed against cell surface antigens**. It is also an example of an immediate hypersensitivity reaction.
2. Antibody-mediated tissue damage: PMNs bind to **antibody-sensitized cells** and destroy the cells by phagocytosis or antibody-dependent cellular cytotoxicity reaction.
3. **Complement-mediated cell lysis:** Antibody-antigen complex on cell surface activates the complement pathway to cause cell lysis.
4. **Incompatible blood transfusions** are examples of this type of hypersensitivity reaction.
5. Damage to sensitized tissue cells causes inflammation, which, in turn, causes damage to normal tissue cells.

### D. Type III Hypersensitivity

1. In type III hypersensitivity (immune complex) reactions, **immune complexes** are deposited on tissues, causing inflammation. This is another example of an immediate hypersensitivity reaction.
2. **Circulating immune complexes:** Large immune complexes are rapidly cleared by mononuclear phagocytes, but smaller immune complexes stay in circulation longer and can be deposited on tissue cells. The immune complexes can activate complement, which can lyse nearby (innocent bystander) cells. The immune complexes can also stimulate **degranulation of granulocytes**, which triggers inflammation and tissue damage.
3. The **heart valves and renal glomeruli** are two sites where immune complexes are often deposited.
4. **Examples**
  - a. **Arthus reaction:** An allergen is injected intradermally.

- b. **Immune complex disorders (serum sickness):** Patients develop antibodies against heterologous serum proteins.
- c. **Glomerulonephritis:** Immune complexes are deposited on renal glomeruli, causing inflammation of the kidney and possibly renal failure.
- d. **Vasculitis:** Inflammation of the blood vessel walls

## E. Type IV Hypersensitivity

1. Type IV hypersensitivity (cell-mediated) reactions are caused by soluble factors or lymphokines released by T cells; antibody and complement are not involved in this reaction. Recruitment and activation of the cells takes 24–72 hours; therefore, this reaction is also referred to as **delayed hypersensitivity**.
2. **Mechanism**
  - a. **Lymphokines** are produced by T cells.
  - b. These chemicals attract macrophages that become activated, causing them to degranulate.
  - c. As more macrophages arrive at the site, ulceration and necrosis occur.
3. **Examples**
  - a. **Tuberculin-type hypersensitivity:** Subcutaneous injection of tuberculosis antigen is used as a diagnostic skin test. Swelling occurring at the site within 24–72 hours indicates previous infection.
  - b. **Contact sensitivity (dermatitis):** Allergens from poison ivy and poison oak cause sensitization, resulting in edema in the skin with the formation of microvesicles and itching on subsequent exposure. Most allergens causing delayed-type hypersensitivity reactions are haptens. They must combine with fatty acids on the skin to be immunogenic.

## VIII. IMMUNE DEFICIENCY

### A. Primary Immune Deficiencies

1. Humoral immune deficiencies
  - a. **Bruton X-linked agammaglobulinemia**
    - 1) A marked deficiency of all classes of immunoglobulins is detected after about 6 months of age.
    - 2) **Recurrent, life-threatening infections** occur with encapsulated bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, manifested as pneumonia, sinusitis, bronchitis, otitis, furunculosis, meningitis, and septicemia.
    - 3) B cells are markedly decreased or absent.
  - b. **Hyper-IgM syndrome**
    - 1) X-linked genetic disease
    - 2) **Serum IgM is increased;** IgG and IgA are markedly decreased or absent.
    - 3) A defect in CD40 ligand on T helper cells prevents class switching from IgM to IgG, IgA, or IgE.
    - 4) Affected individuals are prone to respiratory tract infections.

- 5) Affected individuals often have autoantibodies to platelets, red blood cells, and neutrophils.
  - c. **Selective IgA deficiency**
    - 1) Patients present with small amounts or absence of serum and secretory IgA.
    - 2) Usually caused by a genetic defect or by drugs (phenytoin and penicillin)
    - 3) Anaphylaxis may result if IgA is administered to someone with this deficiency (i.e., blood transfusion).
  - d. **Ataxia-telangiectasia**
    - 1) Autosomal recessive disorder that presents with ataxia, telangiectasia, recurrent sinopulmonary infections, a high incidence of malignancy, and variable immune defects. Patients typically present with an IgA and sometimes IgE deficiency.
    - 2) It is not primarily an immunodeficiency but a defect in a kinase gene that regulates the cell cycle. The B and T helper cells are affected.
2. **Cellular immune deficiencies**
- a. Because T cells are involved in both humoral- and cell-mediated responses, individuals with T helper cell deficiencies can have a severe combined immunodeficiency.
  - b. **Congenital thymic hypoplasia (DiGeorge syndrome)**
    - 1) Symptoms include hypocalcemic tetany, due to underdevelopment of the thymus, and heart disease.
    - 2) Immune defect is variable, from slight decrease in T cells to no T cells in the bloodstream.
    - 3) Patients are very susceptible to opportunistic infections and have a poor prognosis.
3. **Severe combined immune deficiency**
- a. A group of diseases, with different causes, that affect T and B cell function, resulting in a suppression of humoral- and cell-mediated immune responses
  - b. Defects in **adenosine deaminase (ADA)** or **purine nucleotide phosphorylase (PNP)**
    - 1) Absence of these enzymes causes an accumulation of nucleotide metabolites in all cells, which is particularly toxic to T and B cells.
    - 2) Very low number of T cells is present, and children often have an underdeveloped thymus, lack of tonsils or lymph nodes, hypogammaglobulinemia, and lymphopenia.
  - c. **Bare lymphocyte syndrome**
    - 1) With an MHC class II deficiency, T helper cells fail to develop. Patients present with hypogammaglobulinemia and no CMI response.
    - 2) MHC class I deficiency is less severe. There is a loss of CTLs and response to intracellular pathogens.



- d. **Wiskott-Aldrich syndrome**
  - 1) Mutation in the gene that codes for the **Wiskott-Aldrich syndrome protein (WASP)**, a protein involved with cytoskeletal reorganization necessary for delivering cytokines
  - 2) The defect prevents T helper cells from delivering lymphokines to B cells, macrophages, and other target cells.
  - 3) Patients demonstrate eczema, thrombocytopenic purpura, and increased risk of infection. Platelets are small and defective.
- 4. **Complement deficiencies**
  - a. Genetic deficiencies have been described for each of the complement proteins.
  - b. Homozygous deficiencies in any of the early components of the classical complement proteins result in an increase in immune complex diseases.
  - c. Patients with defects in early alternative complement proteins, such as factor D and properdin, are susceptible to infections by *Neisseria meningitidis*.
  - d. Patients with a C3 defect have the most severe clinical manifestations.

## B. Secondary Immune Deficiencies

- 1. Secondary immune deficiencies are due to an underlying cause.
- 2. Transient hypogammaglobulinemia of infancy presents as a decline in serum immunoglobulins during the first few months of life. Individuals eventually produce normal amounts of immunoglobulins.
- 3. **Malignancy**
  - a. Cancers can exert a suppressive effect on the immune system.
  - b. Impairment of antibody production is found in lymphomas, chronic lymphocytic leukemia, and multiple myeloma.
- 4. **Viral disease:** Certain viruses impair the function of the immune system.
  - a. Human immunodeficiency virus
  - b. Epstein-Barr virus
  - c. Cytomegalovirus
- 5. Nutritional deficiencies and defects: Malnutrition and protein-energy malnutrition syndromes (e.g., marasmus)

## IX. HYPERGAMMAGLOBULINEMIA

### A. Polyclonal Hypergammaglobulinemia

- 1. Tremendous amounts of several classes of immunoglobulins to several specific antigens are produced, resulting in a **broad spike in the gamma region on serum protein electrophoresis**.
- 2. **Infectious diseases:** Chronic antigenic stimulation from infectious organisms can create this condition.
- 3. **Inflammatory process:** Many acute-phase proteins are produced during inflammation and can cause a broadening of the alpha-2 peak in serum protein electrophoresis.



4. **Liver disease:** Because of a polyclonal increase in the gamma region and an increase in IgA, the depression between the gamma and the beta regions is absent. As a result, the beta and gamma regions form only one peak on serum protein electrophoresis—beta-gamma bridging, consistent with cirrhosis.

## B. Monoclonal Hypergammaglobulinemia

1. Monoclonal hypergammaglobulinemia is a malignant transformation of a clone of B cells that produce identical antibodies. This causes a narrow peak on serum protein electrophoresis.
2. **Multiple myeloma**
  - a. **Lymphoproliferative disease**, in which plasma cells produce a high concentration of immunoglobulin
  - b. Approximately 50% of patients with multiple myeloma have **Bence Jones protein** (light chain fragment) in their urine.
  - c. **Symptoms:** Weakness, anorexia, weight loss, skeletal destruction, pain, anemia, renal insufficiency, and recurrent bacterial infections
  - d. **Laboratory findings:** Monoclonal gammopathy and plasma cell infiltrate in bone marrow
  - e. **Monoclonal immunoglobulins** (M proteins)
    - 1) Diagnostic of multiple myeloma, Waldenström macroglobulinemia, chronic lymphocytic leukemia, or lymphoma
    - 2) Immunoglobulin type determination is necessary for diagnosis and prognosis.
3. **Waldenström macroglobulinemia**
  - a. **Uncontrolled proliferation of a clone of B cells** that synthesize a homogeneous IgM; cause unknown
  - b. **Hyperviscosity of plasma** causes congestive heart failure, headache, dizziness, partial or total loss of vision, bleeding, and anemia.
  - c. **Symptoms:** Weakness, fatigue, headache, and weight loss
  - d. **Laboratory findings:** A spike in the beta or gamma region on serum protein electrophoresis, increased plasma viscosity, and abnormal accumulation of lymphoid cells in the bone marrow and tissues
4. **Primary amyloidosis**
  - a. An amyloid protein is a nonstructural protein that becomes insoluble after an alteration in its secondary structure. In **amyloidosis**, these proteins accumulate in organs and tissue.
  - b. **Monoclonal plasma cell disorder** in which abnormal immunoglobulin or Bence Jones protein (light fragments) or, less commonly, heavy chain fragment is produced.
  - c. Insoluble proteins are deposited in some of the tissues: Skin, liver, nerves, heart, kidney, etc. This results in progressive loss of organ function.
  - d. **Laboratory findings:** Frequent abnormalities of serum immunoglobulins and presence of Bence Jones proteins

## X. TRANSPLANT IMMUNOLOGY

### A. Types of Grafts

1. **Autograft:** Transfer of tissue from one site to another within an individual
2. **Isograft (syngraft):** Transfer of tissue between genetically identical individuals
3. **Allograft:** Transfer of tissue between two genetically nonidentical individuals of the same species
4. **Xenograft:** Transfer of tissue between two individuals of different species

### B. Graft Acceptance and Rejection

1. Graft acceptance occurs when revascularization and healing lead to a repaired site in about 2 weeks.
2. **Two types of graft rejection**
  - a. **First-set rejection:** The first time a graft is encountered, the immune system attacks and ultimately destroys (rejects) the nonself tissue. This occurs 10–14 days after transplantation.
  - b. **Second-set rejection:** The second time nonself tissue with the same or similar antigens is encountered, it is rejected within 6 days.

### C. Clinical Indications of Graft Rejection

1. **Hyperacute rejection** occurs within 24 hours of transplantation.
  - a. The rejection is caused by a **preexisting antibody** to antigens on the grafted tissue. The tissue never becomes vascularized.
  - b. **ABO blood group antibodies and MHC class I antibodies cause hyperacute rejection.** Donor and recipient must be of the same ABO blood type to avoid rejection.
  - c. Crossmatches are performed on tissue transplants. Serum of the recipient is mixed with mononuclear donor cells, and the mixture is monitored for cytotoxicity.
2. **Acute rejection** occurs within weeks of transplantation. Rejection is due to CMI response; histopathology reveals massive infiltration of lymphocytes and macrophages.
3. **Chronic rejection** occurs months to years after transplantation; mechanisms of rejection include both HMI and CMI.

## XI. TUMOR IMMUNOLOGY

### A. Definitions

1. **Neoplasm:** An abnormal mass of tissue that results from the uncontrolled growth of normal cells even after the growth stimulus is removed
2. **Benign tumor:** Typically a mild and nonprogressive tumor that pushes aside normal tissue, but does not invade it, as the tumor expands

3. **Malignant tumor:** Generally consisting of poorly differentiated cells that grow rapidly and invade surrounding tissue, robbing the normal tissue of nutrients
4. **Metastatic tumor:** Secondary tumor derived from a malignant primary tumor

## B. Tumor-Associated Antigens

1. **Tumor-specific peptides** are intracellular proteins expressed on the surface of a tumor due to interaction with MHC class I and class II molecules. This expression can be chemically induced.
2. **Virus-induced tumors:** Tumors caused by viruses usually have viral antigens on their surface. These cells are sometimes recognized as nonself by the immune system.
3. **Genome-encoded tumor antigens:** When **oncogenes** are deregulated, the protein product can lead to tumor formation. **Proto-oncogenes** are found in nearly all nucleated cells, from yeast to human, and are involved in cell growth. Alteration in gene expression or protein structure can initiate abnormal cell growth.
4. **Oncofetal antigens** are produced during fetal development but present in minute amounts after birth. However, they may become expressed after malignant transformation (e.g.,  $\alpha$ -fetoprotein and carcinoembryonic antigen).

## C. Immunity to Tumors

1. **Natural immunity** to tumors occurs to a limited degree with macrophages and NK cells.
  - a. **Macrophage-mediated cytotoxicity:** Occurs when macrophages come in close contact with tumor cells
  - b. **NK cells:** Approximately 50% of tumors have mutations leading to decreased MHC class I products expressed on their surfaces; this may facilitate killing by NK cells.
2. **Humoral-mediated immunity:** Antibodies can be produced to antigens found on the surface of tumor cells. The tumor cells can then be lysed by complement activation or antibody dependent cellular cytotoxicity (ADCC) reactions involving NK cells, PMNs, and macrophages.
3. **T cell-mediated immunity**
  - a. Cytokines involved in tumor immunity
    - 1) IL-1 activates T cells, B cells, and NK cells and induces a fever.
    - 2)  $\text{TNF}\alpha$  destroys tumor cells.
    - 3)  $\text{INF}\gamma$  is produced by activated T cells and NK cells.
  - b. CTLs can directly lyse tumor cells.

## D. Tumor Markers

1. Tumor markers are glycoproteins found in small amounts in normal serum but elevated in certain types of cancers. They can be used to screen for cancer but

more commonly are used to monitor therapeutic response or to determine tumor burden.

2. **Carcinoembryonic antigen (CEA)**
  - a. CEA levels are used in management of gastrointestinal tumors (colon cancer) and adenocarcinomas of the colon, pancreas, liver, and lung.
  - b. Can also be found in inflammatory bowel disease, ulcerative colitis, Crohn disease, polyps, tumors of the gastrointestinal tract, and cigarette smokers
  - c. The **highest CEA levels** are found in **metastatic disease**.
3.  **$\alpha$ -Fetoprotein (AFP)**
  - a. AFP is produced during embryonic and fetal development. AFP levels are high in patients with hepatocellular carcinoma, hepatoblastoma, and testicular and ovarian cancer.
  - b. Can also be elevated in viral hepatitis, cirrhosis, and ulcerative colitis
  - c. Important marker for **monitoring cancer therapy**
4. **Human chorionic gonadotropin (HCG)**
  - a. Human chorionic gonadotropin is composed of two subunits: alpha and beta (beta-human chorionic gonadotropin;  $\beta$ -HCG). HCG is found in serum and urine during pregnancy.
  - b. HCG may be produced by neoplastic cells of testicular cancer and various other tumors. Levels are useful in evaluating patients with gestational trophoblastic disease, testicular tumors, and ovarian germ cell tumors.
5. **Prostate-specific antigen (PSA)**
  - a. A glycoprotein that dissolves seminal gel formed after ejaculation
  - b. Normal prostate tissue contains PSA, but it is present in extremely low amounts in blood.
  - c. Increased in prostate cancer, benign prostatic hypertrophy, and acute or chronic prostatitis
  - d. Levels correlate with prostate size, stage of prostate cancer, and response to treatment.
  - e. Used to **screen for prostate cancer** in conjunction with a digital rectal examination

## XII. ANTIGEN-ANTIBODY REACTIONS

### A. Antigen-Antibody Interaction

1. Forces that participate in antibody-antigen interaction
  - a. **Electrostatic force or ionic bonding**
    - 1) Positively charged portions of one molecule are attracted to negatively charged portions of another molecule.
    - 2) This bonding is affected by the pH and ionic strength of the environment.
    - 3) Electrostatic force increases as the two molecules get closer together.

- b. **Hydrogen bonding**
    - 1) Hydrogen binds to an electronegative atom such as oxygen or nitrogen.
    - 2) A weak bond, but it contributes greatly to the antigen-antibody interaction
    - 3) Maximum binding strength occurs below 37°C.
  - c. **Hydrophobic bonding**
    - 1) This is the attraction between **nonpolar groups**.
    - 2) The nonpolar groups tend to aggregate to reduce surface area, and this increases the strength of the bond.
  - d. **Van der Waals force:** A weak, attractive force between an electron orbital of one atom and the nucleus of another atom
2. **Affinity**
    - a. The strength of the interaction between a single antibody binding site and a single epitope
    - b. The affinity constant describes whether the antigen-antibody complex is highly complementary, and therefore would bind readily, or not very complementary, and therefore would not bind readily.
  3. **Avidity**
    - a. The affinity for multivalent antigens and multiple antibodies to combine; the extent of binding capacity
    - b. This is greater than the cumulative affinity constants for all antigen-antibody pairs.
  4. **Specificity and cross reactivity**
    - a. **Specificity** refers to the antibody's greatest affinity for a particular antigen.
    - b. **Cross reactivity** occurs when the antibody combines with an antigen that is structurally similar to the immunogen that stimulated the antibody production or the antigen the antibody has the greatest affinity for (i.e., **heterophile antibodies**).

## B. Immunoassays

1. Assays involving antibody-antigen reactions are called immunoassays.
2. **Examples**
  - a. **Precipitation reaction:** Soluble antigen and soluble antibody react to form an insoluble product (precipitate), such as double gel diffusion, radial immunodiffusion, immunoelectrophoresis, immunofixation, nephelometry, and turbidimetry.
  - b. **Agglutination reaction:** Soluble antibody reacts with insoluble antigen or soluble antigen reacts with insoluble antibody. Reactants are made insoluble by combining with latex particles, RBCs, dyes, or liposomes.
  - c. **Labeled reaction:** A label producing a measurable end product is attached to an antibody or antigen. Labels include fluorochromes, enzymes, chemiluminescent molecules, and radionuclides.

- d. Testing serial dilutions of patient sera provides semiquantitative results (titer). A fourfold rise in titer between an acute and convalescent sample is considered clinically significant.

### XIII. PRECIPITATION REACTIONS

#### A. Precipitation

1. **Zone of equivalence:** Maximum precipitation occurs when the concentrations of the antigen and antibody are about equal.
2. **Prozone** occurs when excess amount of antibody is present, and the antigen and antibody do not combine to form precipitates—the complexes remain soluble. This results in a false negative result.
3. **Postzone** occurs when excess amount of antigen is present, and the antigen and antibody do not combine to form precipitates—the complexes remain soluble. This results in a false negative result.

#### B. Types of Precipitation Reactions

1. **Fluid-phase precipitation:** Passive diffusion of soluble antigen and antibody
  - a. **Turbidimetry** is the measurement of light transmitted through a suspension of particles. The formation of immune complexes decreases the amount of light passing through a suspension. The more immune complexes formed and the larger they are, the greater the decrease in light able to pass through.
  - b. **Nephelometry** is a direct measure of light scattered by particles suspended in solution. The scattering of light is proportional to the size and amount of immune complexes formed. **Nephelometry is more sensitive than turbidimetry.**
2. **Precipitation reactions in agar gel**
  - a. Antigen and antibody diffuse through the agar gel and precipitate when they reach the zone of equivalence.
  - b. Molecular size determines the speed of travel through the gel.
  - c. **Double immunodiffusion** (Ouchterlony technique)
    - 1) Antigen and antibody are placed in wells in the gel and diffuse toward each other. When optimum concentrations are met (at the zone of equivalence), a precipitate line forms.
    - 2) Can be used to determine if a specific antibody is present in serum
    - 3) Precipitant lines between adjacent wells of antigen can be reported as identity, partial identity, or nonidentity.
    - 4) **Common errors include** overfilling of wells, irregular well punching, unlevel incubation area, gel drying, increased room temperature, and antigen or antibody contamination by bacteria or fungi.
  - d. **Countercurrent immunoelectrophoresis (CIE)**
    - 1) On an agar gel plate or slide, antigen is added to one well and antibody is added to another well. An electric current accelerates the movement

of the antigen and antibody toward each other, resulting in precipitation sooner than if an electric current is not applied.

- 2) CIE can be used to detect antibodies to infectious agents and microbial antigens. CIE has generally been replaced by easier to perform assays, such as agglutination tests.

e. **Immunofixation electrophoresis**

- 1) Serum, urine, or CSF is electrophoresed. Antisera contained in a cellulose acetate strip is then placed on top of the electrophoresis gel. The antibodies diffuse into the electrophoresis gel and combine with the antigens, forming a precipitate.
- 2) Detects the presence of an immunoglobulin in serum or urine

f. **Rocket immunoelectrophoresis**

- 1) Used to quantify antigens
- 2) Antigens are electrophoresed in agar-containing antibody. A pH is selected so that the antibodies are immobile. The antibody and antigen combine to form precipitates in the shape of a “rocket.”
- 3) The height of the rocket is proportional to the concentration of antigen in the specimen.

## XIV. AGGLUTINATION REACTIONS

### A. General Information

1. **Definition:** Agglutination occurs when particles in suspension clump together due to antibody-antigen interaction.
2. IgM and IgG antibodies participate in agglutination reactions. Because IgM has more antigen binding sites, it agglutinates more quickly.
3. **Comparison of agglutination and precipitation**
  - a. Agglutination uses an antigen or antibody attached to a particle (insoluble), whereas precipitation uses soluble antigens and antibodies.
  - b. Agglutination and precipitation reactions use antigens with at least two antigenic determinants (**epitopes**).
  - c. In agglutination and precipitation reactions, antigen excess can result in a postzone reaction, whereas antibody excess can result in a prozone reaction.
  - d. Agglutination reactions take minutes to hours, whereas precipitation reactions may take hours to days.
  - e. Methods that utilize agglutination reactions are qualitative or semiquantitative, whereas precipitation methods give qualitative, semiquantitative, or quantitative results.

### B. Classification of Agglutination Reactions

1. **Direct agglutination:** This method uses antigens naturally occurring on a particle to demonstrate agglutination (e.g., RBCs in type and crossmatch).



2. **Viral hemagglutination:** This is a naturally occurring process in which a virus (e.g., influenza virus) will agglutinate RBCs by binding to surface receptors.
3. **Passive and reverse passive agglutination**
  - a. **Passive agglutination:** A technique in which soluble antigen is attached to a particle, producing agglutination with a specific soluble antibody
  - b. **Reverse passive agglutination:** A technique in which an antibody is attached to a particle, producing agglutination with a specific soluble antigen
  - c. Particles used include latex, gelatin, resin beads, and RBCs.

## XV. COMPLEMENT FIXATION

### A. Principle

1. **Complement fixation (CF)** assays are sometimes used to detect antibody in patient sera. The serum is mixed with a specific known antigen. If antibody to the antigen is present, an immune complex forms. Complement is added, and if an immune complex is present, it will bind the complement.
2. After the addition of sensitized RBCs, **hemolysis is a negative reaction** (complement is not available), and no hemolysis is a positive reaction, meaning antibody was present in the patient sample.
3. When the antibody and antigen combine in this technique, the complement present in the system also combines with the antigen-antibody complexes and no free complement is available to cause lysis of the sensitized indicator RBCs.
4. If antibody is absent, then complement is free to attach to the sensitized indicator RBCs and causes lysis.

### B. Application

1. CF can be used to detect antibodies to viruses, *Rickettsia*, and fungi. Because IgM is efficient at binding complement, this assay works well for detecting IgM.
2. Although once the reference method for detecting many antibodies, it has largely been replaced by other methods that are easier to perform.

## XVI. LABELED REACTIONS

### A. Immunofluorescence

1. **Definition:** Antibodies labeled with a fluorescent dye are used to detect an antibody or antigen.
2. **Methods**
  - a. **Direct immunofluorescence:** Conjugated (fluorescent labeled) reagent antibody reacts with an antigen in a clinical sample to form an antigen-antibody complex.
  - b. **Indirect immunofluorescent assays:** Antigen reacts with unlabeled antibody forming an antigen-antibody complex that is then complexed with a labeled antihuman antibody, creating an antibody-antigen-antibody “sandwich.”



- c. **Biotin-avidin immunofluorescence:** This is an indirect assay in which the detection system is modified by using a biotin-labeled antibody followed by avidin-labeled fluorochrome. This extra step increases the specificity and sensitivity of the assay.
- 3. Commonly used **fluorochromes** include fluorescein isothiocyanate (FITC), R-phycoerythrin, quantum red, tetramethyl-rhodamine isothiocyanate, Texas red, phycocyanin, acridine orange, and propidium iodide.
- 4. **Antinuclear antibodies (ANAs):** Antibodies to nuclear antigens are present in many systemic autoimmune diseases, such as systemic lupus erythematosus, mixed connective tissue disease, and rheumatoid arthritis. This test is used for diagnosing, developing a prognosis, and monitoring treatment of certain autoimmune diseases.
  - a. **Indirect immunofluorescence** is used for **ANA screening**.
  - b. **Procedure:** Cultured cells on a microscope slide are incubated with patient serum. The cells are washed, then incubated with antihuman immunoglobulin conjugated with fluorescein. The slide is washed again, then viewed using a fluorescent microscope.

## B. Enzyme-Linked Immunosorbant Assays (ELISAs)

- 1. Enzyme-labeled reagents are used to detect antigens or antibodies.
- 2. Enzyme must be stable, specific, and cannot bind to antigen or antibody independently.
- 3. A colorless substrate is metabolized by the enzyme into a colored compound. The intensity of the color is directly proportional to the amount of enzyme present.

# XVII. CELLULAR ASSAYS

## A. Lymphocyte Subsets

- 1. **T cell subsets**
  - a. Enumeration of T cells is important in assessing immune response.
  - b. Monoclonal antibodies are used in conjunction with flow cytometry to identify cell markers such as CD1, CD2, CD3, and CD4.
- 2. **B cell subsets**
  - a. **Classical test:** Labeled antibody to surface membrane immunoglobulin
  - b. Monoclonal antibodies are now used in conjunction with flow cytometry to identify CD19 or CD20.
- 3. **Lymphocyte phenotyping in human immunodeficiency virus (HIV) infection**
  - a. HIV kills T helper cells, and the primary viral receptor for infection is CD4.
  - b. CD4 and CD8 markers are monitored during treatment. If the CD4 count falls below 200/ $\mu$ L, the patient is susceptible to opportunistic infections.
- 4. Other cells identified by flow cytometry and monoclonal antibodies

- a. CD16 on NK cells, macrophages, and neutrophils
- b. CD34 on immature cells
- c. HLA-DR on B cells, monocytes, myeloid cells, and erythroid precursors
- d. Glycophorin A on erythroid cells
- e. CD14 on myelomonocytic cells
- f. CD41 on platelets and megakaryocytes

## B. Assays to Assess Cell Function

1. **Lymphocyte transformation**
  - a. Cells are challenged with antigens and then observed for transformation.
  - b. Normal control cells are stimulated by the antigens while the patient's cells are observed for stimulation.
2. **Mixed-lymphocyte culture**
  - a. Used to detect HLA-Dw on the surface of cells to ensure compatibility of donor cells with recipient cells
  - b. This is critical for bone marrow transplants.
3. **Measurement of immune activation**
  - a. All the events that lead to an immune response
  - b. Measurement includes a WBC count with differential, immunoglobulin levels, and complement levels.
  - c. Signs of immune activation in the patient include swollen lymph nodes, fever, and malaise.
  - d. Cytokines (e.g., IL-2) are measured to detect immune disorders.

## XVIII. STREPTOCOCCAL SEROLOGY

### A. *Streptococcus pyogenes* (Group A Streptococci)

1. *S. pyogenes* causes pharyngitis, pyoderma, puerperal sepsis, and necrotizing fasciitis. It can also produce a toxin that results in scarlet fever.
2. **Post-streptococcal sequelae**
  - a. Antibody-antigen complexes can lead to rheumatic fever and glomerulonephritis. Sequelae are often diagnosed by the antistreptolysin O (ASO), antihyaluronidase, anti-DNase B, or streptozyme tests.
  - b. **Rheumatic fever**
    - 1) **Symptoms:** Carditis, chorea, erythema marginatum, polyarthritis, and/or subcutaneous nodules
    - 2) Occurs 3–4 weeks after infection
    - 3) **Mechanism:** M protein of *S. pyogenes* shares antigenic epitopes with proteins found in synovium, heart muscle, and heart valve, suggesting that the damage is from an autoimmune disease due to **molecular mimicry**.
    - 4) It is most commonly seen between 5 and 15 years of age; although it is rare in the U.S. because of rapid treatment of *S. pyogenes* infections.

c. **Glomerulonephritis**

- 1) **Symptoms:** Proteinuria, hematuria, hypertension, impaired renal function, and edema
- 2) Occurs about 10 days after pharyngitis or 18–21 days after a skin infection
- 3) **Mechanism:** Circulating antigen-antibody complexes are deposited on the glomerular basement membranes, where complement is activated and damage to the membranes results. Platelet aggregation and fibrin and fibrinogen build up, causing capillary obstruction and impaired renal function.

**B. Diagnostic Tests**

1. Culture results yielding beta-hemolytic group A streptococci are most reliable; however, the sequelae are immunologically mediated and do not involve actively growing bacteria.
2. **ASO neutralization test**
  - a. Streptolysin O is a hemolysin produced by most beta-hemolytic group A streptococci.
  - b. Infected individuals produce antibody to streptolysin O.
  - c. The **classic ASO test** is a neutralization assay. Antibodies to streptolysin O prevent hemolysis.
  - d. Serial dilutions of patient serum are prepared. The titer is the last tube with no hemolysis. The result is expressed in **Todd units**, the reciprocal of the original serum dilution (e.g., 1:8 = 8 Todd units).
  - e. **Interpretation:** A fourfold increase in titer between acute and convalescent samples indicates a recent group A streptococcal infection.
  - f. The ASO neutralization test is rarely performed in the U.S.; it has been replaced by other diagnostic methods.
3. **ASO rapid latex agglutination test**
  - a. **Principle:** Latex particles coated with streptolysin O agglutinate when mixed with patient's serum containing ASO antibody.
  - b. **Interpretation:** The following titers are considered indicative of a group A streptococcus infection: preschool children >85, school-age children >170, and adults >85.
4. **Streptozyme**
  - a. Screening test produced by Wampole Laboratories (Cranbury, New Jersey) that detects antibodies to five *S. pyogenes* proteins: DNase B, hyaluronidase, NADase, streptokinase, and streptolysin O
  - b. **Principle:** Streptozyme is a passive hemagglutination assay. Newer methods use latex as the carrier particle. Immunonephelometry assays are also available.
  - c. **Interpretation:** A fourfold rise in titer between acute and convalescent sera is indicative of an infection.

### 5. Anti-DNase B test

- a. Anti-DNase B antibody peaks at 4–6 weeks after group A streptococcal infection and lasts for months.
- b. **Principle:** Today, most methods use latex agglutination or immunonephelometry.
- c. **Interpretation:** The following titers are considered indicative of a group A streptococcal infection: preschool children >60, school-age children >170, and adults >85.

## XIX. SYPHILIS SEROLOGY

### A. Causative Agent

1. *Treponema pallidum* subsp. *pallidum*, a spirochete
2. Transmitted by direct contact (including sexual contact) and across the placenta

### B. Disease Stages

1. **Incubation period:** *T. pallidum* enters the body, reaches the bloodstream, and is disseminated to all organs. This early asymptomatic phase lasts 10 days to 10 weeks.
2. **Primary syphilis**
  - a. The initial lesion is a painless, nonbleeding ulcer called a **chancre**.
  - b. The chancre appears, on average, 2–3 weeks after the initial infection.
  - c. Within 1 week after the chancre appears, lymph nodes enlarge.
  - d. Antibodies are produced 1–4 weeks after the chancre appears.
  - e. Darkfield analysis of lesion demonstrates spirochetes.
3. **Secondary syphilis**
  - a. Symptoms include skin rash, low-grade fever, malaise, pharyngitis, weight loss, arthralgia, and lymphadenopathy. Symptoms last 4–6 weeks.
  - b. Spirochetes are present throughout the body during this stage.
  - c. Ulcers develop on mucous membranes.
  - d. Serologic tests are positive.
4. **Latency**
  - a. Stage of syphilis with no signs or symptoms
  - b. Nontreponemal and treponemal serologic tests are positive.
  - c. **Early latency:** One in four individuals relapses into secondary syphilis.
  - d. **Late latency:** The patient is resistant to reinfection and to relapses.
5. **Tertiary syphilis**
  - a. Symptoms occur 2–40 years after initial infection.
  - b. **Gummas** (syphilis lesions due to hypersensitivity reaction to treponemal antigens) are found throughout the body.
  - c. Syphilitic aortitis, aortic valve insufficiency, and thoracic aneurysm are possible.
  - d. Neurosyphilis can cause blindness and senility.

## 6. Congenital syphilis

- a. *Treponema pallidum* can cross the placenta during any stage of the disease.
  - b. **Infection of the fetus** causes late abortion, stillbirth, neonatal death, neonatal disease, or latent infection.
  - c. The outcome depends on the stage of the mother's disease—primary or secondary syphilis causing the worst outcome—and the age of the fetus at infection.
  - d. If the mother receives treatment during the first 4 months of pregnancy, congenital syphilis is usually avoided.
  - e. Congenital syphilis presents in the neonate as diffuse maculopapular desquamatory rash (particularly around the mouth and on the palms and soles), hemolytic anemia, jaundice, hepatosplenomegaly, abnormal cartilage and bone involvement, and mental retardation.
7. **Diagnosis:** Signs and symptoms, detection of spirochetes in lesion, and positive syphilis serology

## C. Direct Detection

1. **Definitive diagnosis** of syphilis is made by detection of *T. pallidum* in CSF, umbilical cord, or skin or mucous membrane lesions—depending on the stage of the disease.
2. *Treponema pallidum* is detected using **darkfield microscopy or silver stain**.
3. Direct fluorescent antibody-*T. pallidum* (**DFA-TP**) test: A fluorescence-labeled antibody is used to detect *T. pallidum* in lesions.

## D. Serological Tests

### 1. General principles

- a. *Treponema pallidum* infection causes the host to produce nonspecific antibody, **reagin**, and specific treponemal antibodies.
- b. The **nontreponemal antigen tests** detect reagin and are only used for screening because this antibody will cross react with similar antigens present in SLE, autoimmune disease, pregnancy, and some chronic infections such as hepatitis. These conditions can result in biologic false positives.
  - 1) Examples of nontreponemal antigen tests include Venereal Disease Research Laboratory (VDRL), unheated serum reagin (USR), and rapid plasma reagin (RPR) assays.
  - 2) The percentage of false positives in these tests is high (30–40%), so all reactive results must be confirmed using a test that detects antibodies specifically directed at *T. pallidum*, so-called **treponemal antigen tests**.
- c. Treponemal antigen tests use *T. pallidum* cells as the antigen source. These assays are highly specific and include the fluorescent treponemal antibody absorption (FTA-ABS), *T. pallidum*-particulate agglutination (TP-PA), and microhemagglutination *T. pallidum* tests.

2. **VDRL test**

- a. This test measures the antibody (reagin) a patient has formed against cardiolipin, cholesterol, and lecithin.
- b. **Tests are read microscopically for flocculation.** Results are reported as NR (nonreactive), WR (weak reactive), or R (reactive).
- c. The VDRL test is positive 1–3 weeks after the chancre appears.
- d. Mainly limited to use on CSF now, this is the only serologic test approved for testing CSF.

3. **USR test** is a modified VDRL test in which choline-chloride EDTA is added to the VDRL antigen. The addition of this compound allows serum that has not been heat inactivated to be tested.4. **RPR test**

- a. **Macroscopic flocculation**
- b. The assay uses VDRL antigen with **charcoal particles**. The antigen is not attached to the charcoal as in latex agglutination assays. The charcoal is trapped in the flocculation reaction, which allows the reaction to be seen macroscopically.
- c. The test can be qualitative or semiquantitative. Dilutions are made to semiquantify the amount of antibody present.

5. **TP-PA test:** Treponemal antigen is combined with liposomes. If antibodies are present, a mat of agglutination forms in wells of a microtiter plate.6. **FTA-ABS test**

- a. **Principle:** Indirect antibody test
- b. **Nichol's strain** of *T. pallidum* subsp. *pallidum* is affixed into wells of microscope slides.
- c. Patient serum is heat inactivated.
- d. **Nontreponemal antibody is absorbed** from patient serum with a sorbent of **Reiter's strain** of nonpathogenic treponeme.
- e. Sera are placed in the wells of the microscope slide.
- f. FITC-labeled antihuman antibody is added.
- g. Fluorescent reactions are graded 1 to 4+.

**XX. BORRELIA BURGDORFERI SEROLOGY****A. *Borrelia burgdorferi***

- 1. Spirochete
- 2. Causes **Lyme disease**, also referred to as **Lyme borreliosis**

**B. Transmission**

- 1. The microorganism is transmitted to humans in the saliva of a **tick (*Ixodes*)**.
- 2. Because ticks take days to feed, if a tick is removed within 24–36 hours, infection may be prevented.

## C. Lyme Disease

### 1. Early stage

- a. A reddened area on the skin that occurs 2–32 days after being bitten by an infected tick
- b. The reddened area can develop into the classic target or “bull’s eye” rash, called **erythema chronicum migrans**. The rash is present in about 60% of the cases.

### 2. Late stage

- a. The most common symptom of the late stage is arthritis affecting the knees, shoulders, and elbows.
- b. Approximately 15% of patients exhibit **aseptic meningitis**, facial nerve palsy, encephalitis, cranial neuritis, and radiculoneuritis.
- c. Approximately 8% of patients exhibit carditis.
- d. Chronic disease may present as a sclerotic or atrophic skin lesion or a lymphocytoma.

### 3. Antibody response

- a. The first antibody produced in Lyme disease is IgM, which is primarily directed against the outer membrane-associated protein **OspC** and **flagellin** subunits.
- b. Subsequently, in the late stage IgG antibody specific to a number of *B. burgdorferi* antigens is produced.
- c. Antibodies often persist for several years.

## D. Diagnosis

1. Organisms can be cultured; however, this is time consuming and has only moderate sensitivity.
2. **Serology tests**
  - a. Diagnosis can be made if a fourfold increase in titer is detected between an acute serum specimen and a specimen taken 6–8 weeks later (convalescent). A more rapid method is to detect IgM antibodies to *B. burgdorferi* antigens.
  - b. Immunofluorescence and enzyme-linked immunosorbent assays are **screening methods**. Positive specimens should be confirmed by immunoblotting.
  - c. **Immunoblot (Western blot)**
    - 1) **Procedure**
      - a) Antigens are electrophoretically separated on a polyacrylamide gel to form bands.
      - b) The antigenic bands are transferred to an inert membrane filter (e.g., nitrocellulose), then incubated with patient serum.
      - c) After incubation, the membrane is washed and an enzyme-labeled antihuman antibody is added.
      - d) Enzyme substrate is added to detect antigen-antibody reactions.



## 2) Results and interpretation

- a) The **IgM** immunoblot is considered positive if two or more of the following protein bands are reactive: OspC, 39-KDa protein, and the 41-KDa protein.
- b) The **IgG** immunoblot is considered positive if five or more of the following protein bands are reactive: proteins of 18, 21 (OspC), 28, 30, 39, 41, 45, 58, 66, and 93 KDa.

## XXI. RUBELLA SEROLOGY

### A. Virus

1. Single-stranded **RNA** genome
2. Member of the family *Togaviridae*

### B. Clinical Manifestations

1. **Rubella** (German measles)
  - a. Mild, contagious disease characterized by an **erythematous maculopapular rash**
  - b. This virus is spread by droplets through the upper respiratory tract.
  - c. Patients may have a 1- to 5-day prodromal syndrome of malaise, headache, cold symptoms, low-grade fever, and swollen lymph glands at the back of the head.
  - d. Complications include arthritis, encephalitis, and thrombocytopenic purpura.
2. **Congenital rubella**
  - a. Infection of the mother during pregnancy can result in abortion, stillbirth, or birth defects.
  - b. Typical birth defects that occur if the mother is infected during the first 8 weeks of pregnancy include congenital heart disease, cataracts, and neurosensory deafness.
  - c. Mothers infected after 20–24 weeks of pregnancy rarely give birth to babies with birth defects.
  - d. Babies born with congenital rubella syndrome exhibit thrombocytopenia, hepatitis, long-bone lesions, retinitis, encephalitis, interstitial pneumonitis, psychiatric disorders, thyroid disorders, and diabetes mellitus.
3. **Immunologic response**
  - a. As the rash fades, IgG and IgM antibodies can be detected.
  - b. **IgG antibodies** offer **lifetime immunity**, whereas the **IgM antibodies disappear** at about 4–5 weeks after infection.
  - c. Reinfection can occur, but it is asymptomatic.

### C. Immunologic Response

1. **Acute infections**
  - a. A blood specimen should be drawn when the symptoms start and another specimen 5–7 days later.

- b. If at least a fourfold rise in antibody titer is detected and clinical symptoms are present, then a diagnosis of rubella can be made.
  - c. Because of the widespread use of the rubella vaccine, infections in developed countries are rare.
2. **Congenital infections:** Diagnosis can be established if IgM antibodies are present in neonates that have a low birth weight or any symptom of congenital rubella.
3. Most rubella testing in the U.S. is done to determine a woman's immune status against rubella as part of a prenatal examination. The presence of IgG to rubella virus indicates immunity.

#### D. Diagnostic Tests

1. Test methods used include latex agglutination, passive hemagglutination, ELISA, and indirect immunofluorescence.
2. **Hemagglutination inhibition test**
  - a. **Rubella virus agglutinates chick RBCs.**
  - b. Patient serum is combined with rubella antigen. If the patient has antibodies to the rubella antigen, an antigen-antibody complex forms and it does not allow the rubella antigen to agglutinate the indicator chick RBCs.
  - c. **No agglutination** indicates that **antibodies are present.**
3. **Passive agglutination:** Latex particles coated with rubella virus are agglutinated by rubella antibodies, if present.

## XXII. EPSTEIN-BARR VIRUS SEROLOGY

### A. Epstein-Barr Virus (EBV)

1. **DNA virus**
2. Member of the **herpes virus group**
3. Transmission is through saliva.
4. Immunity lasts a lifetime; however, the virus causes latent infections, and infected persons remain carriers for life.
5. Serological tests detect heterophile and virus specific antibodies.

### B. Diseases

1. **Infectious mononucleosis (IM)**
  - a. A disease of the reticuloendothelial system
  - b. Incubation period is 4–7 weeks.
  - c. Onset may be acute or insidious with sore throat, fever, and lymphadenopathy.
  - d. Common findings are **lymphocytosis**, with many **reactive (atypical) lymphocytes**, and enlarged cervical lymph nodes.
  - e. Other signs include fever and malaise.
  - f. The acute phase lasts 2 weeks and requires a long convalescence, up to 1–2 months.

- g. Infected individuals have abnormal white blood cell differentials and sometimes abnormal liver function tests.
- h. Infections usually resolve in 4–6 weeks.
- 2. **Burkitt lymphoma**
  - a. Burkitt lymphoma is a **malignant neoplasm of B lymphocytes**.
  - b. Found in restricted areas of Africa and New Guinea
  - c. Primarily seen in children
- 3. **Nasopharyngeal carcinoma** is a nasopharyngeal squamous cell carcinoma found mainly in southern China.

### C. Laboratory Tests

- 1. **Heterophile antibodies**
  - a. Heterophile antibodies produced in IM react with sheep, beef, ox, and horse RBCs. Approximately 80–85% of adult patients with IM will develop heterophile antibodies, whereas only 50% of children less than 12 years of age will produce heterophile antibodies.
- b. **Paul-Bunnell presumptive test**
  - 1) **Principle:** Heterophile antibodies peak around 2–3 weeks after infection. Serial dilutions of serum are incubated with a 2% suspension of sheep RBCs. Agglutination is a positive reaction.
  - 2) **Results**
    - a) A titer of 28 or less is normal.
    - b) Titer of >56 is suggestive of IM.
  - 3) **Interpretation**
    - a) The Paul-Bunnell test is a screening test to detect heterophile antibodies that is not specific to IM. The test is rarely used today.
    - b) False negative rate is 10–15%.
    - c) The **Davidsohn differential test** differentiates among three different heterophile antibodies based on absorption onto beef RBCs and guinea pig kidney cells. **IM** antibodies are absorbed onto beef RBCs but not guinea pig kidney cells. **Forssman** antibodies are absorbed onto guinea pig kidney cells but not beef RBCs, and **serum sickness** antibodies are absorbed onto both beef RBCs and guinea pig kidney cells.
      - i) Patient serum is mixed with guinea pig antigen or beef RBC antigen; then both mixtures are checked for agglutination with horse RBCs.
      - ii) **IM:** Agglutination of horse RBCs after absorption with guinea pig kidney cells and no agglutination after absorption with beef RBCs
    - d) **MonoSlide Test** (Becton, Dickinson and Company/BBL)
      - i) Patient serum is mixed with a suspension of guinea pig antigen on a cardboard slide.
      - ii) The mixture of patient serum and guinea pig antigen is then mixed with a suspension of horse RBCs and checked for agglutination.

- iii) **Interpretation:** Agglutination is positive for IM heterophile antibodies, and no agglutination is negative.
  - e) **Latex agglutination**
    - i) Latex test: Bovine RBC antigens are absorbed onto latex particles.
    - ii) Agglutination is positive, and no agglutination is negative.
2. **EBV-specific tests**
- a. Can detect anti-viral capsid antigen (VCA), anti-early antigen/diffuse (EA/d), anti-early antigen/restricted (EA/r), and anti-Epstein-Barr nuclear antigen (EBNA) antibodies
  - b. **Interpretation:** VCA antibodies peak 3–4 weeks following infection, and IgM is not detectable in 12 weeks.
  - c. EBV-specific antibodies can be detected by ELISA, immunofluorescent assay, and immunoblot techniques.

## XXIII. VIRAL HEPATITIS SEROLOGY

### A. Hepatitis Testing

- 1. Testing for antibodies and antigens in patient sera can determine the responsible virus, stage of infection, and immune status of patient.
- 2. The most widely used test method is ELISA.

### B. Hepatitis A

- 1. Hepatitis A virus (HAV): Member of the family *Picornaviridae*
- 2. **Epidemiology**
  - a. Transmission by **fecal-oral route**
  - b. Epidemics occur through fecal contamination of food or water.
- 3. **Clinical manifestations**
  - a. Infections may be asymptomatic or symptomatic; **infections in children are usually asymptomatic.**
  - b. Incubation period is 10–50 days.
  - c. **Symptomatic infections**
    - 1) **Symptoms** include fever, anorexia, vomiting, fatigue, abdominal pain, and malaise. Patient may become jaundiced. Symptoms are more severe in pregnant women.
    - 2) Recovery occurs in 2–4 weeks.
    - 3) Mortality rate is 0.1%, and chronic disease rarely occurs.
    - 4) Inactivated **vaccines**, first developed in 1995, are recommended for travelers, drug abusers, and children.
  - d. **Laboratory tests**
    - 1) **Aspartate aminotransferase (AST)** and especially **alanine amino-transferase (ALT)** levels are increased and peak before jaundice occurs.
    - 2) Other findings include **hyperbilirubinemia**, decreased albumin, tea-colored urine, and pale-colored stools.

- 3) Paired sera (acute collected at onset of symptoms and convalescent 3–4 weeks later) are analyzed for an increase in anti-HAV antibodies. Alternatively, a single acute sample with a higher titer of IgM compared to IgG is considered diagnostic of an acute infection.
- 4) Anti-HAV antibodies are present at onset of symptoms and for years afterward.

### C. Hepatitis B

1. Hepatitis B virus (HBV)
  - a. Partially double-stranded DNA
  - b. Member of the family *Hepadnaviridae*
  - c. **Dane particle:** Complete HBV virus (42 nm) that causes infection
2. **Epidemiology**
  - a. The virus is transmitted via mucous membranes (e.g., sexual contact) or wounds contacting contaminated blood and body fluids, or parenterally. Parenteral infection occurs through transfusion of contaminated blood products, hemodialysis, intravenous drug use, contaminated needle sticks, tattooing, acupuncture, or ear piercing.
  - b. **High-risk groups** for acquiring HBV infection include intravenous drug users, men who have sex with men, hemodialysis patients, and healthcare workers.
3. **Clinical manifestations**
  - a. Incubation period is 50–180 days.
  - b. **Symptoms** develop abruptly and include fever, anorexia, vomiting, fatigue, malaise, jaundice, and arthralgia.
  - c. **Long clinical course:** Acute infection can last up to 6 months. Most patients recover within 6 months.
  - d. Approximately 5% of infected patients develop a chronic infection, in which the patient remains **hepatitis B surface antigen (HBsAg)** positive.
  - e. If chronic infections are active, severe damage to the liver occurs, which can result in liver cirrhosis or hepatocellular carcinoma.
  - f. All chronic carriers shed virus.
  - g. A recombinant HBV vaccine is recommended for healthcare workers. The Advisory Committee for Immunization Practices now recommends routine vaccination for all children in the U.S.
4. **Laboratory tests**
  - a. The **first marker that appears** at the end of the incubation period is HBsAg. The concentration of the surface antigen continues to rise and peaks about midway through the acute infection. Presence of this antigen indicates infectivity.
  - b. Soon after HBsAg is detected in the blood, **hepatitis Be antigen (HBeAg)** appears. HBeAg peaks at about the same time as the surface

antigen. HBeAg disappears about two-thirds of the way through the acute infection phase.

- c. The next marker to appear is **antibody to hepatitis B core** (anti-HBc), which begins to rise a couple weeks into the acute infection. Anti-HBc peaks at the end of the acute infection stage after HBsAg is no longer detectable and before **antibody to hepatitis B surface antigen** (anti-HBs) can be detected. This period is referred to as the “core window.”
- d. The anti-HBc IgM antibody peaks a few weeks after the acute infection stage, then disappears in about 6 months during recovery. Anti-HBc IgG will persist for several decades.
- e. **At the end of the acute stage, anti-HBe begins to rise** and peaks about 2–16 weeks later. The concentration of this antibody decreases slightly during a person’s lifetime but never disappears.
- f. **The last marker to appear is anti-HBs.** It appears at the end of the acute stage and the beginning of the recovery stage. Its concentration peaks, then plateaus during recovery and never disappears. Presence of this antibody indicates immunity.
- g. **In chronic infections,** patients do not produce detectable levels of anti-HBs, and HBsAg persists. These patients become chronic carriers of the virus and are at risk for cirrhosis and hepatocellular carcinoma.

## D. Hepatitis C

1. Hepatitis C virus (HCV)
  - a. Single-stranded RNA virus
  - b. Member of the family *Hepacivirus*
2. **Epidemiology**
  - a. Parenteral transmission is most common.
  - b. Sexual and perinatal transmission of the virus is less common.
3. **Symptoms**
  - a. Causes either acute or chronic disease
  - b. The **incubation period** is 2–26 weeks.
  - c. **Acute infections** are asymptomatic or mild—nausea, vomiting, abdominal pain, fatigue, malaise, and jaundice.
  - d. Approximately 50–80% of cases become chronic, with 25% leading to cirrhosis.
  - e. About 20% of cirrhosis cases lead to cancer.
4. **Laboratory tests**
  - a. Anti-HCV is diagnostic of HCV infection.
  - b. Anti-HCV IgM does not distinguish between acute and chronic disease because both IgM and IgG antibodies are detectable for years.
  - c. ELISA tests have false positive results, so the best test to use for diagnosis is an immunoblot assay.

### E. Delta Hepatitis

1. Hepatitis D virus (HDV)
  - a. Unclassified, single-stranded RNA virus
  - b. Requires HBsAg from HBV infection to replicate and infect host
2. **Epidemiology**
  - a. Occurs worldwide
  - b. Transmission is via the parenteral and transmucosal routes.
3. **Symptoms**
  - a. **Coinfection** occurs when patients acquire HBV and HDV infections simultaneously.
  - b. **Superinfection** occurs in patients with an established HBV infection who acquire HDV infection; superinfections can occur and progress to chronic HBV/HDV infection.
  - c. Patients with **chronic** HBV/HDV infection have poor prognoses because of severe liver damage, inflammation, and cirrhosis.
  - d. Vaccination against HBV also prevents HDV.
4. **Laboratory Tests**
  - a. Only HBsAg positive patients are tested for HDV.
  - b. **HDV-Ag** is the **first marker** to appear, detectable about 1–4 days before symptoms start.
  - c. IgM anti-HDV appears next followed by low levels of IgG anti-HDV.
  - d. The switch to high levels of IgG anti-HDV indicates past HDV infection.

## XXIV. HUMAN IMMUNODEFICIENCY VIRUS SEROLOGY

### A. Human Immunodeficiency Virus (HIV)

1. Member of the family *Retroviridae*
2. **HIV causes acquired immunodeficiency syndrome (AIDS).**
3. There are **two serogroups**. HIV-1 is the predominant strain, and it is found worldwide. HIV-2 is limited primarily to West Africa.
4. **HIV-1 has three subtypes:** M, N, and O. M is the major subtype.

### B. HIV Replication

1. **HIV binds to the CD4 molecule** on T helper cells, monocytes, macrophages, and other cells. Secondary receptors (co-receptors) are also important in viral binding. T helper cells are the primary target.
2. HIV penetrates the plasma membrane of the cell, and the viral RNA is released.
3. The RNA is transcribed to DNA by the activity of the viral enzyme **reverse transcriptase**. Viral DNA is then inserted into the host cell's DNA by viral **integrase**.
4. The viral DNA is transcribed into mRNA, which is then translated into viral proteins. Mature viruses leave the host cell by budding.



5. The replication process kills the infected cell and leads to a diminishing number of T helper cells.

### C. Immune Response and HIV

#### 1. Serologic effects

- a. Antibodies to HIV generally appear about 12 weeks after infection. These are the first antibodies detected by ELISA and Western blot assays.
- b. Neutralizing antibodies, antibodies able to interfere with infection of host cells, appear about 1 year after infection. Although these neutralizing antibodies can interfere with viral replication, they do not seem to play a major role in protection.
- c. HIV is able to escape the immune response by undergoing **antigenic variation**.

#### 2. Effect on T cells

- a. As the disease progresses, there is a **depletion of CD4+ T helper cells**. The immune deficiency worsens as more T helper cells are killed by the virus.
- b. HIV compromises the immune response by destroying T helper cells. These cells are key players in both humoral and cellular immune responses.
- c. **The ratio of CD4 to CD8 cells is reduced from 2:1 (normal).**

#### 3. Additional effects

- a. Decreased natural killer cell activity
- b. Defective chemotaxis in monocytes and macrophages
- c. Enhanced release of interleukin-1 and cachectin by monocytes

### D. Epidemiology

1. HIV-1 is transmitted by unprotected sex, contaminated blood or blood products, contaminated needles, or perinatally.
2. In the U.S., AIDS is the number one cause of death for people between 20 and 35 years of age.

### E. Symptoms

1. Initially, infected persons (acute phase) will be asymptomatic or can have minor symptoms resembling IM.
2. The virus continues to replicate rapidly in the lymphoid tissue. This stage is referred to as **clinical latency**.
3. As the number of T cells begins to decrease, the patient develops a number of infections caused by **opportunistic pathogens**: *Candida*, herpes simplex virus, cytomegalovirus, etc. This stage has been referred to as **AIDS-related complex (ARC)**.
4. Final stage (full-blown AIDS) includes T cell depletion resulting in severe opportunistic infections and cancers, such as esophageal candidiasis, cryptococcosis, systemic cytomegalovirus and herpes simplex virus

infections, *Pneumocystis jiroveci* pneumonia, and Kaposi's sarcoma (caused by human herpes virus 8).

5. CD4+ T cell counts and presence of a variety of opportunistic infections are used to stage the severity of the disease.

#### F. Laboratory Tests

1. ELISA tests are used to detect antibodies to HIV and HIV antigen. Repeatedly positive samples must be confirmed by a Western blot or immunofluorescent test.
2. **The Western blot assay is the confirmatory serological test for HIV.** Two of the three bands must appear for a Western blot to be considered positive: p24, gp41, or gp120/160.
3. Genetic probes can detect replicating viruses.
4. Reverse transcriptase—polymerase chain reaction assays detect nucleic acid gene sequences in HIV-1 and HIV-2.
5. The indirect immunofluorescence assay is used to detect HIV antigen in infected cells. This can also be used as a confirmatory test.



# review

# questions

## INSTRUCTIONS

Each of the questions or incomplete statements that follows is comprised of four suggested responses. Select the *best* answer or completion statement in each case.

1. Color Plate 21■ depicts a monomeric immunoglobulin molecule. The portion of the molecule indicated by the dotted red circle and the red arrow is called the
  - A. Fab fragment
  - B. Fc fragment
  - C. Heavy chain
  - D. Hinge region
2. A hapten is
  - A. Half of an immunoglobulin molecule
  - B. A carrier molecule for an antigen that is not antigenic alone
  - C. An immunoglobulin functional only in the presence of complement
  - D. A determinant capable of stimulating an immune response only when bound to a carrier
3. Which of the following is characteristic of B cells?
  - A. Phagocytic
  - B. Participate in antibody-dependent cellular cytotoxicity (ADCC) reactions
  - C. Contain surface immunoglobulins
  - D. Secrete the C5 component of complement
4. A lymphokine is
  - A. A soluble mediator produced by granulocytes and affecting lymphocytes
  - B. A soluble mediator produced by lymphocytes
  - C. A soluble mediator produced by plasma cells
  - D. An antibody that reacts with lymphocytes

5. Monocytes and macrophages play a major role in the mononuclear phagocytic system. For an antibody-coated antigen to be phagocytized, what part of the antibody molecule fits into a receptor on the phagocytic cell?
  - A. Fc region
  - B. Fab region
  - C. Hinge region
  - D. Variable region
6. Cell-mediated immunity is primarily mediated by
  - A. B cells
  - B. T helper cells
  - C. Plasma cells
  - D. Dendritic cells
7. The HLA complex is located primarily on
  - A. Chromosome 3
  - B. Chromosome 6
  - C. Chromosome 9
  - D. Chromosome 17
8. HLA antigens are found on
  - A. All nucleated cells
  - B. Red blood cells only
  - C. Solid tissue only
  - D. White blood cells only
9. Which of the following is more likely to be diagnostic of an acute infection?
  - A. A total acute antibody titer of 2 followed by a convalescent titer of 16
  - B. A total acute antibody titer of 80 followed by a convalescent titer of 40
  - C. A total antibody titer of 80
  - D. An IgG antibody titer of 80
10. A young woman shows increased susceptibility to pyogenic infections. Upon assay, she shows a low level of C3. Which of the following statements is probably *true*?
  - A. She has an autoimmune disease with continual antigen-antibody activity causing consumption of C3.
  - B. She has DiGeorge syndrome.
  - C. She has decreased production of C3.
  - D. She may produce an inactive form of C2, a precursor of C3.
11. What is the predominant type of antibody found in the serum of neonates born after full-term gestation?
  - A. Infant IgA
  - B. Infant IgG
  - C. Infant IgM
  - D. Maternal IgG
12. An important part of the nonspecific immune response is(are)
  - A. B cells
  - B. Basophils
  - C. Complement cascade
  - D. Cytotoxic T lymphocytes
13. The major class of immunoglobulin found in adult human serum is
  - A. IgA
  - B. IgE
  - C. IgG
  - D. IgM
14. Which class of immunoglobulin possesses delta heavy chains?
  - A. IgA
  - B. IgD
  - C. IgE
  - D. IgG

15. Which class of immunoglobulin possesses 10 antigenic binding sites?
  - A. IgA
  - B. IgD
  - C. IgG
  - D. IgM
16. Color Plate 22 ■ represents a dimeric IgA molecule. The structure printed in red and indicated by the red arrow is called the
  - A. J-piece
  - B. Hinge region
  - C. Heavy chain
  - D. Light chain
17. Which class of immunoglobulin binds to basophils and mast cells to mediate immediate hypersensitivity reactions?
  - A. IgA
  - B. IgD
  - C. IgE
  - D. IgG
18. Type I hypersensitivity is
  - A. Associated with complement-mediated cell lysis
  - B. Due to immune complex deposition
  - C. Mediated by activated macrophages
  - D. An immediate allergic reaction
19. When performing the enzyme-multiplied immunoassay technique (EMIT), how is the ligand in the patient's serum detected?
  - A. Agglutinates by binding to antibody-coated latex beads
  - B. Binds to enzyme-labeled antibody
  - C. Competes with enzyme-labeled antigen for binding to a specific antibody
  - D. Forms antibody-antigen complex and precipitates
20. Severe combined immunodeficiency (SCID) is an
  - A. Immunodeficiency with decreased B cells and neutrophils
  - B. Immunodeficiency with lymphocytopenia and eosinophilia
  - C. Immunodeficiency with decreased or dysfunctional T and B cells
  - D. Immunodeficiency with decreased lymphocytes and decreased complement concentration
21. An example of immune injury due to the deposition of antigen-antibody complexes is
  - A. Acute glomerulonephritis
  - B. Bee-sting allergy
  - C. Contact dermatitis
  - D. Penicillin allergy
22. The serologically detectable antibody produced in rheumatoid arthritis (RA) is primarily of the class
  - A. IgA
  - B. IgE
  - C. IgG
  - D. IgM
23. In bone marrow transplantation, immunocompetent cells in the donor marrow may recognize antigens in the recipient and respond to those antigens. This phenomenon is an example of
  - A. Acute rejection
  - B. Chronic rejection
  - C. Graft versus host disease
  - D. Hyperacute rejection
24. Multiple myeloma is a
  - A. Lymphoproliferative disease of T cells
  - B. Cancer of plasma cells characterized by increased antibody concentration
  - C. Lymphoproliferative disease resulting in a decrease in antibody production
  - D. Cancer of monocytes characterized by increased kappa and lambda chain synthesis

25. Which one of the following describes a direct immunofluorescence assay?
  - A. Conjugated reagent antigen reacts with antibodies to form antigen-antibody complexes
  - B. Antigens react with unlabeled antibody forming antigen-antibody complexes that attach to labeled antibodies
  - C. A dye is attached to a molecule and it reacts with an immune complex to produce a color
  - D. Conjugated reagent antibody reacts with antigen to form antigen-antibody complexes
26. In individuals allergic to pollen, hyposensitization protocols may be initiated. These individuals receive injections of
  - A. Allergen
  - B. Pooled human antisera
  - C. Monoclonal antibody directed against human T cells
  - D. Monoclonal antibody directed against human B cells
27. After exposure to antigen, the first antibodies that can be detected belong to the class
  - A. IgA
  - B. IgE
  - C. IgG
  - D. IgM
28. Corneal tissue may be transplanted successfully from one patient to another because
  - A. The cornea is nonantigenic
  - B. Corneal antigens do not activate T cells
  - C. Anticorneal antibodies are easily suppressed
  - D. The cornea occupies a privileged site not usually seen by the immune system
29. A kidney transplant from one identical twin to another is an example of a(n)
  - A. Allograft
  - B. Autograft
  - C. Isograft
  - D. Xenograft
30. In Bruton disease, measurement of serum immunoglobulins would show
  - A. Elevated levels of IgE
  - B. Elevated levels of IgG
  - C. Normal levels of IgG and IgM but reduced levels of IgA
  - D. The absence of all immunoglobulins
31. Diagnosis of group A streptococci (*Streptococcus pyogenes*) infection is indicated by the presence of
  - A. Anti-protein A
  - B. Anti-DNase B
  - C. Anti-beta-toxin
  - D. C-reactive protein
32. A molecule found in human serum sometimes used as a tumor marker is
  - A.  $\alpha$ -Fetoprotein
  - B. HBsAg
  - C. Biotin
  - D. CD1
33. Which cell is the principal source of interleukin 2?
  - A. B cell
  - B. T cell
  - C. Monocyte
  - D. Plasma cell
34. Diagnostic reagents useful for detecting antigen by the coagglutination reaction may be prepared by binding antibody to killed staphylococcal cells via the Fc receptor of staphylococcal protein A. The class of antibody bound by this protein is
  - A. IgA
  - B. IgD
  - C. IgG
  - D. IgM

35. A major advantage of passive immunization compared to active immunization is that
  - A. Antibody is available more quickly
  - B. Antibody persists for the life of the recipient
  - C. IgM is the predominant antibody class provided
  - D. Oral administration can be used
36. The strength with which a multivalent antibody binds a multivalent antigen is termed the
  - A. Affinity
  - B. Avidity
  - C. Reactivity
  - D. Valence
37. How does the secondary humoral immune response differ from the primary response?
  - A. The lag phase (the time between exposure to immunogen and production of antibody) is longer in the secondary immune response.
  - B. IgM is the predominant antibody class produced in the secondary immune response.
  - C. The antibody levels produced are higher in the secondary immune response.
  - D. Cytotoxic T lymphocytes play an important role in the secondary response.
38. After activation of the complement system, leukocytes and macrophages are attracted to the site of complement activation by
  - A. C1
  - B. C5a
  - C. C8
  - D. IgM
39. The type of immunity that follows the injection of an immunogen is termed
  - A. Artificial active
  - B. Natural active
  - C. Artificial passive
  - D. Innate
40. The type of immunity that follows the injection of antibodies synthesized by another individual or animal is termed
  - A. Artificial active
  - B. Natural adaptive
  - C. Artificial passive
  - D. Natural passive
41. Innate immunity includes
  - A. Anamnestic response
  - B. Antibody production
  - C. Cytotoxic T cell activity
  - D. Phagocytosis by polymorphonuclear cells
42. The agglutination pattern shown in Color Plate 23■ was observed while performing an antibody titration. This agglutination pattern is an example of
  - A. A prezone reaction
  - B. A prozone reaction
  - C. A postzone reaction
  - D. Incomplete complement inactivation
43. The antibody most frequently present in systemic lupus erythematosus is directed against
  - A. Surface antigens of bone marrow stem cells
  - B. Surface antigens of renal cells
  - C. Nuclear antigen
  - D. Myelin
44. The rapid plasma reagin assay for syphilis does not need to be read microscopically because the antigen is
  - A. Cardiolipin
  - B. Complexed with latex
  - C. Complexed with charcoal
  - D. Inactivated bacterial cells



45. The Venereal Disease Research Laboratory (VDRL) test for syphilis is classified as a(n)
  - A. Agglutination reaction
  - B. Flocculation reaction
  - C. Hemagglutination reaction
  - D. Precipitation reaction
46. One cause of a false-positive VDRL test is
  - A. Brucellosis
  - B. *Treponema pallidum* infection
  - C. Rocky Mountain spotted fever
  - D. Systemic lupus erythematosus
47. The portion of an antigen that binds to an antibody or T cell receptor is called a(n)
  - A. Allergen
  - B. Avidin
  - C. Epitope
  - D. Valence
48. Identical antibodies produced from a single clone of plasma cells describes
  - A. Reagin
  - B. Cold agglutinins
  - C. Heterophile antibodies
  - D. Monoclonal antibodies
49. IgM antibodies react well in complement fixation (CF) tests. Because of this, CF tests for antibodies should
  - A. Be positive early in the course of the disease
  - B. Be useful in identifying antibodies responsible for a delayed hypersensitivity reaction
  - C. Be useful in identifying antibodies responsible for anaphylactic reactions
  - D. Detect transplacental antibodies
50. Which of the following serologic tests is commonly performed by an immunofluorescence method?
  - A. Anti-HBs
  - B. Antinuclear antibody (ANA)
  - C. Antistreptolysin O (ASO)
  - D. C-reactive protein (CRP)
51. The Fab portion of an antibody
  - A. Binds T cell receptor
  - B. Consists of two light chains only
  - C. Consists of two heavy chains only
  - D. Contains the hypervariable region
52. In the enzyme-linked immunosorbent assay (ELISA), the visible reaction is due to a reaction between
  - A. Enzyme and antibody
  - B. Enzyme and substrate
  - C. Fluorescent dye and antigen
  - D. Latex particles and antibody
53. Elevated IgE levels are typically found in
  - A. Type I hypersensitivity reactions
  - B. Type II hypersensitivity reactions
  - C. Type III hypersensitivity reactions
  - D. Type IV hypersensitivity reactions
54. Loss of self-tolerance results in
  - A. Autoimmune disease
  - B. Graft-versus-host disease
  - C. Immunodeficiency
  - D. Tumors
55. A human cell with CD8 on its surface is most likely a
  - A. B cell
  - B. Monocyte
  - C. T helper cell
  - D. Cytotoxic T cell
56. Which of the following statements about immunoglobulin light chains is *true*?
  - A. Each immunoglobulin monomer has either one kappa or one lambda chain.
  - B. There are two types: kappa and lambda.
  - C. They consist of constant regions only.
  - D. They form part of the Fc fragment.

57. Which of the following statements applies to the Fc fragment of an immunoglobulin molecule?
  - A. It consists of the entire heavy chain.
  - B. It contains the variable region of the heavy chain.
  - C. It contains the antigen binding sites of the molecule.
  - D. It is the region of the molecule that binds to receptors on various white blood cells.
58. Monoclonal antibodies are produced by
  - A. Cultured T cells
  - B. Human plasma cells
  - C. Mouse plasma cells
  - D. Hybridomas
59. Antibodies that bind to the same epitope are of the same
  - A. Allotype
  - B. Autotype
  - C. Idiotype
  - D. Isotype
60. Skin testing is a useful diagnostic tool in a number of disorders, such as tuberculosis. Which of the following statements about skin testing is *true*?
  - A. A positive test depends on preformed antibody.
  - B. Reactivity to a particular antigen may be transferred from one individual to another by sensitized lymphocytes.
  - C. The intensity of the response correlates directly with the clinical activity of the disease.
  - D. The maximum response will occur immediately.
61. The activity of natural killer (NK) cells
  - A. Does not require previous exposure to an antigen
  - B. Involves phagocytosis and killing of bacteria
  - C. Requires interaction with cytotoxic T cells
  - D. Requires interaction with B cells
62. Interaction between B and T helper cells involves
  - A. MHC II molecule on B cell binding to MHC I molecule on the T cell
  - B. MHC II molecule on B cell binding to CD3 on the T cell
  - C. Foreign antigen on B cell binding to T cell receptor
  - D. CD3 molecule on B cell binding to T cell receptor
63. Which of the following is a characteristic of T cells?
  - A. Synthesize antibody
  - B. Mature in the thymus
  - C. Able to bind unprocessed antigen
  - D. Primarily protect against extracellular parasites
64. The primary mechanism responsible for pathology in systemic lupus erythematosus is
  - A. Allergic reaction to foreign molecules
  - B. Antibodies directed against self antigens
  - C. Polyclonal activation of cytotoxic T cells
  - D. Lack of intracellular killing after neutrophil phagocytosis of bacteria
65. Which complement protein is present in the greatest concentration in human serum?
  - A. C1
  - B. C2
  - C. C3
  - D. C4

66. An autoimmune disease causing destruction of pancreatic cells can result in
- Hashimoto disease
  - Multiple sclerosis
  - Myasthenia gravis
  - Type 1 diabetes
67. An Ouchterlony gel diffusion plate is depicted in Color Plate 24■. The center well contains antibody, and the peripheral wells contain antigens labeled 1 through 4. What is the relationship between the antigens in wells 2 and 3?
- 2 is part of 3.
  - 3 is part of 2.
  - They are identical.
  - They are unrelated.
68. An Ouchterlony gel diffusion plate is depicted in Color Plate 24■. The center well contains antibody, and the peripheral wells contain antigens labeled 1 through 4. What is the relationship between the antigens in wells 2 and 4?
- Cannot be determined.
  - They are identical.
  - They are unrelated.
  - They react incompletely with the antibody.
69. Which of the following complement proteins is part of the membrane attack complex?
- C1
  - C3
  - C4
  - C5
70. Which of the following is characteristic of contact hypersensitivity reactions?
- Caused by preformed IgE antibody
  - Characterized by infiltration of neutrophils into the area of reaction
  - The primary symptoms often occur in the respiratory tract.
  - Usually due to a hapten
71. Which of the following statements about the test for C-reactive protein (CRP) is *true*?
- It correlates with neutrophil phagocytic function.
  - It is an indicator of ongoing inflammation.
  - It is diagnostic for rheumatic fever.
  - Levels decrease during heart disease.
72. In the classical pathway of complement activation,
- C3 is activated by binding C-reactive protein
  - The sequence of activation is C1, C2, C3, C4
  - C1q is activated by the presence of a single Fab region
  - Activation by antibody requires one IgM or two IgG molecules
73. The alternative complement pathway
- Can be activated by bacterial capsule polysaccharides
  - Uses C5b as a C3 convertase
  - Bypasses steps C3 through C5
  - Is inactivated by properdin
74. A cut on a person's finger becomes contaminated with the bacterium *Staphylococcus aureus*. The first response by the immune system consists of activity of
- B cells
  - Monocytes
  - Neutrophils
  - T cells
75. Incompatible blood transfusions are examples of
- Type I hypersensitivity reactions
  - Type II hypersensitivity reactions
  - Type III hypersensitivity reactions
  - Type IV hypersensitivity reactions

76. A soluble antigen and soluble antibody reacting to form an insoluble product describes
- A. Agglutination reactions
  - B. Heterophile reactions
  - C. Labeled reactions
  - D. Precipitation reactions
77. Which of the following is an example of a treponemal antigen test used for the diagnosis of syphilis?
- A. CRP
  - B. RPR
  - C. VDRL
  - D. FTA-ABS
78. A serum sample is positive for HBsAg. This result indicates that the person from whom the serum was taken
- A. Had a hepatitis B infection in the past but overcame the infection
  - B. Has either active or chronic hepatitis B infection
  - C. Was immunized recently against the hepatitis B virus
  - D. Is not infectious for the hepatitis B virus
79. What is the indicator system used in the complement fixation test?
- A. Sensitized sheep red blood cells
  - B. Fluorescent-labeled antihuman globulin
  - C. Enzyme-labeled antihuman globulin
  - D. Guinea pig complement
80. The isotype of an immunoglobulin antibody
- A. Is defined by the heavy chain
  - B. Is defined as different alleles of the same antibody type (e.g., IgG)
  - C. Is constant for all immunoglobulins of an individual
  - D. Is the variation within the variable region
81. A patient report states the presence of serum antibodies to OspC. What disease does the patient most likely have?
- A. Syphilis
  - B. Strep throat
  - C. Lyme disease
  - D. Rubella
82. Patient serum is mixed with a suspension of guinea pig antigen. When the sample is then mixed with horse red blood cells, agglutination occurs. This is suggestive of an infection caused by
- A. *Borrelia burgdorferi*
  - B. Hepatitis B virus
  - C. Hepatitis C virus
  - D. Epstein-Barr virus
83. Hashimoto disease is an autoimmune disease primarily involving the
- A. Kidneys
  - B. Liver
  - C. Lungs
  - D. Thyroid gland
84. Rheumatic fever sometimes occurs after group A streptococcal infections. In this condition, an autoimmune response attacks the tissue of the heart valves. This phenomenon is an example of
- A. Epitope spreading
  - B. Molecular mimicry
  - C. Polyclonal B cell activation
  - D. Preferential activation of T helper cells
85. "Superantigens" are toxins produced by some strains of *Staphylococcus aureus* and group A streptococci and cause damage by
- A. Molecular mimicry
  - B. Polyclonal T cell activation
  - C. Lysing white blood cells and platelets
  - D. Lysing red blood cells

86. The first serologic marker to appear in patients with acute hepatitis B virus infection is
- A. Anti-HB
  - B. Anti-HBc
  - C. Anti-HBe
  - D. HBsAg
87. A living donor is being sought for a child who requires a kidney transplant. The best odds of finding an MHC-compatible donor occur between the child and
- A. A sibling (brother or sister)
  - B. An unrelated individual
  - C. The child's father
  - D. The child's mother
88. Cells that can act as antigen-presenting cells for exogenous antigens include
- A. All nucleated cells
  - B. Endothelial cells
  - C. B lymphocytes
  - D. T lymphocytes
89. In patients with human immunodeficiency virus infection, immune status can be monitored by measuring the ratio of
- A. CD3+ cells to CD8+ cells
  - B. CD4+ cells to CD8+ cells
  - C. Lymphocytes to monocytes
  - D. T cells to B cells
90. Why does vaccination against hepatitis B virus (HBV) also prevent hepatitis D virus (HDV) infections?
- A. An immunogen from HBV in the vaccine is also associated with HDV.
  - B. The HBV vaccine induces formation of heterophile antibodies that cross react with HDV.
  - C. The HBV vaccine stimulates liver cells to produce antiviral molecules active against all hepatitis viruses.
  - D. HDV requires the host to be concurrently infected with HBV.
91. B lymphocytes and T lymphocytes are derived from
- A. Hematopoietic stem cells
  - B. Macrophages or monocytes
  - C. Mucosa-associated lymphoid tissue
  - D. The fetal liver
92. Contact dermatitis is mediated by
- A. B lymphocytes
  - B. T lymphocytes
  - C. Macrophages
  - D. Polymorphonuclear cells
93. In a competitive radioimmunosorbent test (RIST), what does a high signal suggest?
- A. The patient sample has a low concentration of IgE.
  - B. The patient sample has a low concentration of IgM.
  - C. The patient sample has a high concentration of IgE.
  - D. The patient sample has a high concentration of total antibody.
94. An antibody titration is depicted in Color Plate 25■. In this titration, a 0.2 mL aliquot of a patient's serum sample was added to 0.8 mL of saline, and this mixture was placed into tube #1. A 0.5 mL sample was removed from tube #1 and placed into tube #2, containing 0.5 mL of saline. This procedure was repeated through tube #10. The dilutions were assayed for antibody to an infectious agent. How should the antibody titer be reported?
- A. 256
  - B. 512
  - C. 640
  - D. 1280
95. In a chemiluminescent immunologic assay, what is the signal detected?
- A. Light
  - B. An electric signal
  - C. A purple-colored compound
  - D. A yellow-colored compound

96. A 28-year-old female complains to her family physician of abdominal pain, loss of appetite, and low-grade fever. Physical examination reveals abdominal tenderness and a low-grade fever. Her physician orders a hepatitis profile and obtains the results below.

Anti-HAV	Nonreactive
Anti-HBc	Reactive
Anti-HBs	Nonreactive
HBsAg	Reactive
HBeAg	Reactive
Anti-HCV	Nonreactive

Which of the following is the most likely conclusion?

- A. Acute HAV infection
- B. Acute HBV infection
- C. Chronic HBV infection
- D. Immunity to HBV due to past infection

97. An 11-year-old female presents with fever, sore throat, lethargy, and tender cervical lymphadenopathy. Relevant findings include splenomegaly and lymphocytosis, with many large reactive (atypical) lymphocytes. A heterophile antibody test was negative. Further laboratory results were as follows:

	IgG Titer	IgM Titer
Cytomegalovirus (CMV)	20	0
Epstein-Barr virus (EBV) VCA	0	80
Mono spot	0	0

What conclusion can be made concerning the diagnosis?

- A. Acute CMV infection
  - B. Acute EBV infection
  - C. Chronic CMV infection
  - D. Chronic EBV infection
98. A male infant had been well until about 5 months of age, at which time he was diagnosed as having otitis media and bronchitis caused by *Haemophilus influenzae*. Over the next several months he presented with streptococcal pneumonia several times. At 10 months of age a serum protein electrophoresis showed a virtual lack of gamma globulins. Quantitative serum levels were as follows: 75 mg/dL IgG and undetectable levels of IgM, IgA, and IgE. There were a normal number of T cells, and they exhibited normal mitogen stimulation. What disease does this child most likely suffer from?
- A. Combined immunodeficiency
  - B. DiGeorge syndrome
  - C. Iatrogenic immunodeficiency
  - D. X-linked agammaglobulinemia

99. A 25-year-old male presents to his family physician complaining of fatigue, diarrhea, and weight loss of a few months duration. On physical examination the patient is found to have a fever and abdominal discomfort. Laboratory results indicate a white blood cell count of  $14.3 \times 10^9/\text{L}$  (reference range  $4.8\text{--}10.8 \times 10^9/\text{L}$ ). Assays for HBsAg and anti-HCV are negative. An ELISA test for antibodies to the human immunodeficiency virus (HIV) performed on the patient's serum is found to be reactive. What step should be taken next?
- A. Call the physician with the HIV result.
  - B. Repeat the HIV ELISA test on the sample.
  - C. Test the patient's serum for anti-HBs.
  - D. Contact the patient to collect a second sample.
100. A 38-year-old woman visited her physician because of fatigue, fever, and joint pain (proximal interphalangeal, wrist, and knee joints). She also noticed sensitivity to the sun and reported having a rash following recent exposure. The physician noted a rash over her nose and cheeks. Laboratory results included white blood cell count  $5.5 \times 10^9/\text{L}$  (reference range  $4.8\text{--}10.8 \times 10^9/\text{L}$ ) and red blood cell count  $4.5 \times 10^{12}/\text{L}$  (reference range  $4.0\text{--}5.4 \times 10^{12}/\text{L}$ ). Urinalysis results were within reference ranges, except for 4+ protein and 1+ RBCs, 0–3 hyaline casts/lpf and 0–1 RBC cast/lpf on microscopic examination. Which of the following tests would be most helpful in diagnosing this patient's condition?
- A. Anti-nuclear antibody
  - B.  $\alpha$ -Fetoprotein
  - C. Anti-streptolysin O
  - D. Hepatitis profile





# answers & rationales

1.

**A.** The basic structure of all immunoglobulins is two light chains joined to two heavy chains by disulfide bonds. The amino terminus of both the heavy and light chains, together, constitutes the Fab fragment (fragment of antigen binding). The carboxy-terminus of the heavy chains constitutes the Fc fragment. The hinge region is the area at the center of the “Y,” near the carboxy-terminus of the light chains.

2.

**D.** Haptens are substances that are not immunogenic by themselves. These molecules are not large or complex enough to stimulate the immune system. When bound to a carrier, they are capable of stimulating a specific immune response.

3.

**C.** B cells carry surface immunoglobulins that react to a specific antigen. The antigen can then be internalized processed and presented to an appropriate T helper cell. B cells are not phagocytic, nor do they participate in antibody-dependant cellular cytotoxicity (ADCC) reactions. Complement proteins are secreted by hepatocytes.

4.

**B.** Lymphokines are soluble mediators of immune reactions. They are produced most often by T lymphocytes. Antibodies are produced by plasma cells.

5.

**A.** The Fc region of an IgG molecule fits into an Fc receptor (FcR) on macrophages and monocytes. The Fc receptor binds to specific amino acid residues in the Fc region of the immunoglobulin. The variable region of immunoglobulin binds to the antigen.

6.

**B.** T helper cells are the primary mediators of cell-mediated immunity (CMI). They secrete several different lymphokines that stimulate a number of other cells, such as cytotoxic T lymphocytes and monocytes. B cells differentiate into plasma cells during a humoral-mediated immune response. Dendritic cells are important antigen presenting cells, but they are not the primary mediators of a CMI response.

7.

**B.** The HLA system is part of a larger region known as the major histocompatibility complex. It is located on chromosome 6. The region is located on the short arm of the chromosome. Chromosome 15 contains one HLA gene, B2M.

8.

**A.** Human leukocyte antigens (HLAs) are a group of antigens originally described on human white cells. It is now known that they are found on all nucleated cells of the body, including solid tissue cells. HLAs are not found on red blood cells.

9.

**A.** The most significant indicator of acute or recent infection is the presence of a rising antibody titer. A fourfold or greater rise in titer, from 2 to 16, is significant. Even relatively high antibody titers of IgG may indicate past infection. IgM is produced first following infections, so a high IgM titer is also suggestive of an acute infection.

10.

**C.** C3 may be decreased due to a genetic defect that causes deficient production. In certain autoimmune disorders, such as systemic lupus erythematosus, continual complement activation leads to low levels; however, susceptibility to pyogenic infections is not a feature of autoimmune diseases. DiGeorge syndrome is a deficiency in T cells, and complement protein C2 is not a precursor of C3.

11.

**D.** Antibody production is immunogen induced. Because the fetus develops in a sequestered site, it makes very little immunoglobulin. Maternal IgG crosses the placenta and is the primary antibody found in infant's circulation.

12.

**C.** Important parts of an animal's nonspecific immune response include phagocytosis, inflammation, and complement activation. In a nonspecific immune response, the animal responds in much the same way to all invaders. B cells and cytotoxic T lymphocytes responded to specific antigens and are, therefore, involved in the specific immune response. Basophils are involved in type I hypersensitivity reactions.

13.

**C.** Immunoglobulin G is the predominant class of immunoglobulin found in serum. It accounts for approximately 80% of the total serum immunoglobulin. The normal range is 800–1600 mg/dL.

14.

**B.** The heavy chains divide human immunoglobulin molecules into separate classes and subclasses. The delta ( $\Delta$ ) heavy chain corresponds to IgD. The remaining classes IgA, IgE, IgG, and IgM correspond to  $\alpha$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively.

15.

**D.** The IgM molecule is a pentamer that contains 10 binding sites. However, the actual valence falls to 5 with larger antigen molecules, probably because of steric restrictions. IgA, IgG, IgD, and IgE monomers each have two antigenic binding sites.

16.

**A.** IgA is found in mucous secretions as a dimer stabilized by the J-piece. IgA is synthesized locally by plasma cells and dimerized intracellularly. IgM is also held together by a J-piece, but it exists as a pentamer.

---

**17.**

**C.** Mast cells and basophils have surface receptors (FcεRI) for the Fc portion of IgE. When IgE molecules, attached to the surface of mast cells and basophils, bind the allergen they are specific for, this triggers the cells to degranulate, producing the symptoms of immediate type I hypersensitivity. The main function of IgE appears to be the ability to trigger an immune response, thereby recruiting plasma factors and effector cells to areas of trauma or parasite infection.

---

**18.**

**D.** Type I hypersensitivity reactions occur immediately after second exposure to an allergen. On the first, or primary, exposure, IgE specific to the allergen is produced. The IgE binds to Fc receptors on the surface of basophils and mast cells. Immune complexes and complement are not involved in the response.

---

**19.**

**C.** In the EMIT, a ligand (antigen) in a sample competes with an enzyme-labeled ligand for binding to a specific antibody. The labeled ligand is designed so that following antibody binding, the enzyme is inactive. As the ligand concentration in the test sample increases, more enzyme-labeled ligand remains unbound, resulting in greater enzyme activity.

---

**20.**

**C.** SCID is defined as a condition in which adaptive immune responses (i.e., cell-mediated and humoral-mediated immune responses) do not occur because of a lack of T and B cell activity. A number of genetic defects can lead to this condition. Children born with SCID need to live in a sterile environment, and they have a short life expectancy.

---

**21.**

**A.** Acute glomerulonephritis is caused by the presence of a soluble circulating antigen (Ag) that provokes and combines with antibody (Ab). As these Ag-Ab complexes reach a critical size, they are deposited in the glomerular membranes of the kidney. Upon deposition, an acute inflammatory reaction occurs because of complement activation. Bee-sting and penicillin allergies are examples of IgE-mediated anaphylactic reactions. Contact dermatitis is mediated by T cells, not antibody.

---

**22.**

**D.** Rheumatoid factor (RF) is an immunoglobulin that reacts with antigenic determinants on an IgG molecule. Although they may be of several types, the one that is easily serologically detectable is IgM. This is because of the agglutination activity of the molecule. RF tests are commonly used in the diagnosis of rheumatoid arthritis.

---

**23.**

**C.** Bone marrow transplants by their nature contain immunologically competent cells: B cells and T cells in particular. Unless the transplanted marrow is HLA-matched perfectly to the donor, the immunocompetent cells in the transplant will recognize and react against the nonself HLAs of the recipient's tissues. This phenomenon is known as graft-versus-host disease, because the graft attempts to reject its host. Acute rejection, chronic rejection, and hyperacute rejection are examples of mechanisms a recipient's immune system uses to reject a graft.

---

**24.**

**B.** Plasma cells are normally end-stage cells; they live a few days and die. During multiple myeloma, plasma cells become cancerous and continue to secrete antibody. The cells also secrete excess light chains that can be found in the urine; these proteins are called Bence Jones proteins.

25.

**D.** In a direct immunofluorescence assay, a fluorescent molecule is linked to an antibody. This complex is often called a conjugate. Clinical material is fixed onto a microscope slide, and the conjugate is added. After a wash step, the slide is examined with a microscope using UV light. If antigen specific to antibody was present in the clinical specimen, fluorescence will be seen.

26.

**A.** Hyposensitization, allergy injections, involves the administration of gradually increasing concentrations of an allergen. The goal is for the patient to become tolerant of the allergen and no longer exhibit an allergic response to the allergen. It is hypothesized that patients will ultimately develop high concentrations of IgG to the allergen, blocking IgE from binding and thereby preventing the allergic reaction.

27.

**D.** The first B cells to respond to antigen differentiate into plasma cells that produce IgM antibody. Later in the immune response, stimulated B cells undergo a phenomenon called “class switching” and begin to produce antibodies of the IgG, IgA, and IgE classes. High concentration of IgM in patient serum is indicative of a recent infection.

28.

**D.** Corneas are readily transplanted from one individual to another. This is because the cornea is nonvascularized and is a sequestered site. Thus the immune system of the host does not “see” the cornea and recognize it as foreign.

29.

**C.** Identical twins have the same genetic makeup. Grafts between them would be isografts or syngeneic grafts. Autografts are transplantations

from one site to another in the same individual. Xenograft refers to transplantation between different species. Transplantation between two nonidentical individuals of the same species is called an allograft.

30.

**D.** Bruton disease is a congenital form of agammaglobulinemia. It is a sex-linked phenomenon that affects males. Because B cells are not produced, affected males have levels of IgA, IgD, IgE, and IgM undetectable by routine assays. IgG may be absent or present at very low levels.

31.

**B.** The serological diagnosis of group A streptococcal infection can be made by demonstrating anti-DNase B. The antistreptolysin O (ASO) assay can also be used; however, ASO response is poor in skin infections. C-reactive protein is an acute-phase protein indicating inflammation.

32.

**A.**  $\alpha$ -Fetoprotein (AFP) and carcinoembryonic antigen (CEA) are oncofetal antigens that become expressed after malignant transformation. Approximately 70% of patients with primary hepatoma have elevated levels of AFP. However, the major use of determining AFP levels is in monitoring patients undergoing cancer treatment.

33.

**B.** Interleukin 2 (IL-2) is a lymphokine produced by activated T helper cells. IL-2 principally affects T cells, including the cell that released IL-2, acting on its target cells via the IL-2 receptor. This receptor is not present on resting cells.

---

34.

**C.** Staphylococcal protein A binds only the IgG class (subclasses IgG1, IgG2, and IgG4) of immunoglobulin. Binding occurs via the Fc portion of the antibody molecule, leaving the Fab portion available to bind antigen in an immunologic assay. Binding of the Fab portion to test antigen causes agglutination of the staphylococcal cells (coagglutination).

---

35.

**A.** In passive immunization, preformed antibody is delivered to the recipient, making the antibody available immediately. In active immunization, a period of days is required before antibody production occurs. Passive immunity is short-lived, in contrast to the possibly lifelong persistence of actively induced antibody. Because passive immunization involves the transfer of antibodies, the oral route cannot be used—antibodies are digested in the gastrointestinal tract. The antibodies administered by passive immunization consist largely of the IgG class.

---

36.

**B.** “Avidity” is used to describe the strength of binding between a multivalent antibody and multivalent antigen. “Affinity” describes the bond between a single antigenic determinant and an individual combining site. “Valence” refers to the number of antigenic determinants on an antigen.

---

37.

**C.** The secondary immune response is characterized by the predominance of IgG over IgM. In addition, because of the formation of memory cells following the primary response, the secondary response occurs much more quickly and strongly. This is the basis for immunization as a protection against various infectious diseases. Cytotoxic T lymphocytes are not involved in humoral immunity.

---

38.

**B.** The complement-activation product C5a is chemotactic for neutrophils and macrophages. Neither C1 nor C8 (which occur in the plasma before complement activation) possesses such chemotactic properties. IgM antibody, although capable of activating complement by the classical pathway, is not a chemotactic factor for phagocytic cells.

---

39.

**A.** Active immunity follows exposure to an antigen that stimulates the recipient to develop his or her own immune response. Vaccines are an example of artificial immunity in that the animal was exposed to the immunogen by the actions of a healthcare provider (unnatural). Surviving infections can result in natural active immunity. Protection is due to the formation of memory cells.

---

40.

**C.** Artificial passive immunity results following the injection of antibody synthesized by another individual or animal. This type of immunity is only temporary but may be very important in providing “instant” protection from an infectious agent before the recipient would have time to actively synthesize antibody. The injected antibodies are treated as foreign proteins and are eventually cleared from the body.

---

41.

**D.** Innate, or nonspecific, immunity refers to host defenses that are in general present at birth and do not require immunogen stimulation. Phagocytosis of bacteria by polymorphonuclear cells is an example. Cytotoxic T cell activity is part of the adaptive cell-mediated immune response, and antibody production is the mechanism of protection in the adaptive humoral-mediated immune response.



42.

**B.** Prozone occurs when an extremely high titer of antibody is present. In the first tubes of the titration, not enough antigen is present to allow for cross-linking and lattice formation. The antibody effectively blocks all the antigen sites present, so agglutination does not occur. Complement is not involved in antibody titration.

43.

**C.** Antinuclear antibody (ANA) is the most consistent feature of systemic lupus erythematosus (SLE). Although renal or nerve pathology may occur, that pathology is secondary to deposition of antigen-antibody complexes and subsequent activation of complement proteins. Bone marrow stem cells are not involved in the pathology of SLE.

44.

**C.** The rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) tests use a cardiolipin antigen. However, in the RPR test, charcoal particles are included with the antigen. When antibody in the patient sample combines with the antigen, the charcoal is trapped in the immune complex, allowing the reaction to be read macroscopically.

45.

**B.** The cardiolipin antigen is particulate, not soluble, in the VDRL test. However, the particles are too small to make macroscopic agglutinates when combined with antibody. This type of reaction is called a flocculation reaction and needs to be read with low-power microscopy.

46.

**D.** Patients with connective tissue disorders such as systemic lupus erythematosus may show a false positive reaction in the VDRL test. Other causes of false positives include rheumatic fever,

infectious mononucleosis, malaria, and pregnancy. *Treponema pallidum* subsp. *pallidum* is the causative agent of syphilis.

47.

**C.** Antigens can have multiple epitopes. Each epitope can be unique, binding an antibody with a different idiootype. "Valence" refers to the number of epitopes on an antigen.

48.

**D.** Monoclonal antibodies are derived from a single clone of plasma cells. Plasma cells are fused with a cancerous myeloma cell. Reagin has two meanings: it can refer to the antibody produced during syphilis or it can refer to IgE. Cold agglutinins are antibodies that agglutinate in cold temperatures (e.g., 4°C). Heterophile antibodies are antibodies produced following exposure to an immunogen that are able to bind a similar but different molecule.

49.

**A.** In most infections, IgM antibodies will develop first followed by IgG, which develop higher titers and are longer lasting. Anaphylactic reactions are caused by IgE antibody. Delayed hypersensitivity reactions are caused by T cells. Transplacental antibodies belong to the IgG class. IgG antibodies, although they can be detected by complement fixation (CF), do not fix complement efficiently.

---

**50.**

**B.** Testing for antinuclear antibodies (ANAs) is commonly performed by the immunofluorescence method—using fluorescein-conjugated antihuman antibody to detect patient antibody bound to nuclear components of test cells. Anti-streptolysin O tests are performed with red cells or, more recently, by latex agglutination. Anti-HBs assays are generally performed by ELISA, and C-reactive protein assays are generally performed by latex agglutination, turbidimetry, or nephelometry.

---

**51.**

**D.** The Fab portion of an antibody contains the hypervariable region. This portion of the molecule has a variable sequence of amino acids that affects the three-dimensional structure of the molecule and, therefore, determines the specificity (idiotype) of the antibody. This region contains the amino terminal portion of the two light chains and the two heavy chains.

---

**52.**

**B.** The indicator system in an ELISA test consists of an enzyme and its substrate. If the enzyme-labeled antibody has complexed with the immobilized antigen, the addition of substrate will produce a colored end product. Alkaline phosphatase is an enzyme frequently used in ELISA tests. Latex particles, fluorescent dyes, and red blood cells are not used in ELISA tests but in other test methodologies.

---

**53.**

**A.** Elevated IgE levels are found in type I hypersensitivity reactions. The antibody binds via the Fc portion of the molecule to Fc receptors on mast cells and basophils. When the attached antibody binds its specific allergen, the cell degranulates.

---

**54.**

**A.** The immune system recognizes host cells as self and is tolerant to antigens on those cells. The loss of tolerance will result in an autoimmune disease in which the immune system mounts an immune response against self cells. Graft-versus-host disease occurs when a bone marrow graft is incompatible with the host tissue and attacks the host.

---

**55.**

**D.** The CD8 molecule is found primarily on cytotoxic T lymphocytes. T helper cells possess CD4 on their surface, as do several other cell types. CD3 is a marker found on most T cells.

---

**56.**

**B.** Light chains are of two distinct types: kappa and lambda. Either type may combine with any of the heavy chains, but in any one molecule, only one type is found. Each immunoglobulin monomer contains two light chains, either kappa or lambda. They extend into the Fab, or antigen-binding, site. This half of the chain is highly variable, whereas the carboxy-terminal portion of the molecule is a constant region.

---

**57.**

**D.** The Fc (crystalline) fragment of an immunoglobulin is produced by papain digestion of an immunoglobulin monomer. The Fc portion of antibodies binds to specific Fc receptors on the surface of some white blood cells. Only part of the heavy chain is found in the Fc fragment. The Fab fragment contains the antigen-combining sites of both the heavy chains and the light chains.



58.

**D.** A monoclonal antibody is produced by a single cell or clone. Plasma cells obtained from an immunized animal and subsequently fused with myeloma cells result in a hybrid myeloma or hybridoma that will indefinitely secrete a specific antibody. Hybridomas have been prepared from mouse and human plasma cells fused with myeloma cells. T cells do not produce antibodies.

59.

**C.** Idiotypic of an antibody refers to the antigen specificity of the molecule. The isotype is the different classes and subclasses of antibodies (e.g., IgG, IgM, etc.). "Allotype" refers to different alleles of the same isotype. Genetically different individuals will produce antibodies of the same isotype, but they would have a different allotype.

60.

**B.** Skin testing is based upon the presence of T cells sensitized to antigen. Their activation produces a delayed hypersensitivity reaction, which reaches its peak in about 48 hours. There is no correlation of the amount of the reaction with clinical disease. If the sensitized T cells are transferred from one individual to another, the recipient individual will manifest the same delayed hypersensitivity as the donor.

61.

**A.** The natural killer (NK) cells destroy target cells through an extracellular nonphagocytic mechanism. NK cells are part of the host's innate resistance and, therefore, do not need previous exposure to an antigen to be active. They also do not need interaction with B or cytotoxic T cells.

62.

**C.** B cells have the ability to present antigen (immunogen) to T helper cells. This interaction involves several surface molecules. The antigen

is complexed with MHC II on the surface of the B cell. CD4 on the T cell interacts with MHC II, whereas the T cell receptor binds the antigen.

63.

**B.** T cells are produced in the bone marrow and mature in the thymus. Plasma cells, not T cells, produce antibody, and T cells can only react to antigen processed by an antigen-presenting cell. The cell-mediated immune response, which requires the activity of T cells, is primarily helpful in fighting against intracellular parasites.

64.

**B.** Antibodies directed against self antigens form immune complexes and activate complement. Circulating immune complexes, composed of nuclear antigen and antinuclear antibody, deposit in various organ systems, activate complement, and produce organ pathology. T cells are not directly involved in this process. Allergens, phagocytosis, and killing of ingested bacteria by neutrophils do not play a role in the pathogenic process.

65.

**C.** Complement protein C3 has a serum concentration of about 1300  $\mu\text{g/mL}$ , which makes it the complement protein present in the greatest concentration. The second highest concentration of complement protein is C4 (600  $\mu\text{g/mL}$ ). C3 is cleaved into fragments: C3a and C3b.

66.

**D.** Destruction of the beta cells in the pancreas results in type 1 diabetes. An autoimmune response destroys the insulin-producing cells. The immune response is probably due to molecular mimicry. Cytotoxic T cells and antibodies directed against an infectious agent cross react to the beta cells.

---

**67.**

**D.** The two antigens are not related. There are two different antibodies that are able to react with the two antigens, forming precipitin lines that cross. If the antigens were identical, a smooth curve precipitation line would have formed.

---

**68.**

**B.** When two antigens are identical, a smooth curved line of precipitation is formed between them. In the diagram, the antigen in well 2 is identical to one of two antigens in well 1. The same antigen in well 1 is identical to antigen in well 4. Therefore, it follows that antigens 2 and 4 are identical.

---

**69.**

**D.** The membrane attack complex forms following the binding of C5 to a biologic membrane. The complex is formed by the sequential addition of C6, C7, C8, and C9. When C5–C8 complex with C9, a tubule is formed that bridges the cell membrane.

---

**70.**

**D.** Contact dermatitis is a cell-mediated hypersensitivity reaction. The offending substance is typically a hapten that combines with a carrier molecule on the skin surface. The hapten-carrier complex is recognized by T cells. IgE mediates immediate hypersensitivity reactions such as hay fever and some forms of asthma.

---

**71.**

**B.** CRP is an acute-phase reactant. Although it is elevated in inflammation, its presence is not diagnostic for any one disease, such as rheumatic fever. It does not correlate with antibody levels or with neutrophil phagocytic function. CRP levels are sometimes elevated during heart disease.

---

**72.**

**D.** Complement attaches to the Fc portion of the antibody molecule. At least two Fc binding sites are required for C1q to attach. Therefore, activation requires two IgG molecules or a single molecule of IgM, which is a pentamer. The C proteins were named in order of discovery. The correct reaction sequence is C1, C4, C2, C3. As the last step of this reaction sequence, C3 is split into C3a and C3b.

---

**73.**

**A.** The alternative pathway for complement activation is a more nonspecific defense mechanism, in that it does not require the presence of antibody for activation. It can be activated by a variety of substances, including complex polysaccharides found in bacterial capsules and cell walls. These materials activate C3 directly. Properdin protein stabilizes some of the active complement proteins, and C4b2a is a C3 convertase.

---

**74.**

**C.** The first response to invading bacteria is mounted by the innate immune system. The innate immune system, although it lacks the specificity of the adaptive immune system, is nonetheless effective at handling many invading bacteria. The first response by the innate immune system consists of an influx of neutrophils into the tissue invaded by bacteria. Monocytes and macrophages, although they are phagocytic cells and part of the innate immune system, play only a minor role in the initial response to bacterial invasion.

---

**75.**

**B.** Incompatible blood transfusions are examples of a type II hypersensitivity reaction. These reactions are characterized as the antigen being a part of a cell. Antibody binds to the antigen, complement is activated, and the red blood cells are lysed.

76.

**D.** Precipitation reactions involve both soluble antigens and antibodies. These reactions are typically detected in agarose gels. With agglutination reactions, one of the reactants is soluble and the other is insoluble. A reactant is made insoluble by combining with a carrier particle such as latex beads.

77.

**D.** The fluorescent treponemal antibody absorbance (FTA-ABS) test is often used as a confirmatory test for syphilis. *Treponema pallidum* subsp. *pallidum*, the causative agent of syphilis, is the source of the antigen. The rapid plasma reagin (RPR) and Venereal Disease Research Laboratory (VDRL) are diagnostic tests for syphilis that use nontreponemal antigen. C-reactive protein (CRP) is not involved in syphilis testing.

78.

**B.** Hepatitis B surface antigen (HBsAg) is a marker for active or chronic infection by the hepatitis B virus; it indicates ongoing viral replication. A person positive for this marker is infectious. If the person had overcome a past infection, he or she would have antibody to the surface antigen (anti-HBs) but not the surface antigen. Immunization causes formation of anti-HBs antibody, and the surface antigen would not be present in serum.

79.

**A.** The first step in the complement fixation test, the test system, involves the reaction of antibody in the patient's serum to the corresponding antigen in the presence of guinea pig complement. If antibody-antigen binding occurs, complement will bind to the immune complexes. The second step is the addition of sensitized sheep red blood cells (the indicator system). If complement bound to the immune complexes in the first step, it is not available to lyse the sensitized red blood cells. If antibody was not present in the patient

sample, complement will not bind to the immune complexes, and it will be free to lyse the sensitized cells.

80.

**A.** The isotype of an antibody is determined by which heavy chain is present. The term "idiotype" refers to the variable region of an immunoglobulin molecule. The variable region is the portion of immunoglobulin that binds antigen. Every immunoglobulin with a given antigenic specificity has a unique idiotype.

81.

**C.** IgM antibody to OspC is an important early marker in the diagnosis of Lyme disease. This antibody, along with several others, is often detected by Western blot. Antibodies to p35, p39, and the flagellin subunits p37 and p41 are also useful in diagnosing this disease.

82.

**D.** The Davidsohn differential test can be used to detect heterophile antibodies produced during infectious mononucleosis. These antibodies are not adsorbed by guinea pig antigens. Therefore, the antibodies are available to agglutinate horse red blood cells. Forssman heterophile antibodies are absorbed by guinea pig antigens and would not agglutinate the horse red blood cells.

83.

**D.** Hashimoto disease is a type of thyroiditis due to an autoimmune disease. Patients produce autoantibodies and T cells that respond to thyroid antigens. This results in inflammation and swelling of the thyroid gland (goiter). The autoantibody blocks the uptake of iodine, which results in a decrease in the production of thyroid hormones (hypothyroidism).

84.

**B.** Group A streptococci contain antigenic determinants that are similar to antigenic determinants found on heart valve tissue in some individuals. The immune response occurring during the course of a group A streptococcal infection may be extensive enough to include an immune-mediated attack on the heart valves—rheumatic heart disease. “Molecular mimicry” is the term given to this phenomenon, whereby an immune response directed against one antigen may be extended to include activity against closely related antigens.

85.

**B.** Some strains of *Staphylococcus aureus* and group A streptococci produce toxins that have the properties of “superantigens.” Superantigens react with T cells directly without processing by an antigen presenting cell. These toxins can stimulate many T cells, rather than only those T cells bearing T cell receptors specific for the bacterial toxins. The result is a massive T cell response, leading to the release of cytokines and resulting in disease entities known as toxic shock syndrome (in the case of *S. aureus* infection) and toxic shock–like syndrome in the case of group A streptococci.

86.

**D.** HBsAg is the first serologic marker occurring in patients with hepatitis B virus infection. The antigen appears about 3–5 weeks before symptoms appear. About 2–4 weeks later, anti-HBc, primarily of the IgM class, begins to appear.

87.

**A.** Because the human leukocyte antigen (HLA) system is extremely polymorphic, the odds are greatly against finding an HLA-compatible donor in unrelated individuals. The genes coding for HLA antigens are inherited from one’s parents and are expressed co-dominantly. Between an

offspring and either parent, there is, statistically, a 25% chance of an HLA match. Between siblings, there is a 50% chance of an HLA match.

88.

**C.** Exogenous antigens are nonself antigens derived from infectious agents or immunizing preparations. Exogenous antigens are processed for presentation to specific T cells by specialized cells collectively referred to as antigen-presenting cells (APCs). APCs for exogenous antigens include B cells, macrophages, monocytes, and dendritic cells.

89.

**B.** Human immunodeficiency virus preferentially infects T helper cells, which are positive for the surface marker CD4. As the infection progresses, the number of CD4+ cells in the peripheral bloodstream decreases. CD8 is a marker found on another subset of T cells, cytotoxic T cells. The reference ratio of CD4:CD8 cells is 2:1. A decrease in the ratio indicates a decline in immune function.

90.

**D.** HDV requires HBsAg produced by HBV-infected cells. HDV, therefore, requires the host to be concurrently infected with HBV. The HBV vaccine prevents HBV infection and also HDV infection.

91.

**A.** The stem cells of the bone marrow give rise to both T and B cells, as well as other cells in the bloodstream. Macrophages and monocytes also arise from hematopoietic stem cells, but they do not differentiate into lymphocytes. Mucosa-associated lymphoid tissue contains mature lymphocytes, particularly B cells, but is not the source of lymphocytes. The fetal liver is a maturation site for B lymphocytes during fetal life but is not the source of those lymphocytes.



92.

**B.** Contact dermatitis is a delayed-type hypersensitivity reaction mediated by T cells. Antibody is not involved in this type of hypersensitivity, so B cells play no role in it. Neither macrophages nor neutrophils are involved in this type of hypersensitivity.

93.

**A.** The competitive RIST assay is used to determine the concentration of total IgE. Patient sample containing IgE is mixed with labeled IgE. Both labeled and unlabeled IgE are captured by antihuman IgE. After a wash step, the signal from the label is detected. A high signal indicates a low concentration of unlabeled IgE from the patient sample.

94.

**C.** The titer of this assay is the reciprocal of the highest dilution demonstrating the desired result, in this case tube #8. The dilution is determined as shown below.

Dilution for tube #1: 0.2 mL serum in a total volume of 1.0 mL = 1:5 dilution.

Dilutions in succeeding tubes: 0.5 mL diluted serum in a total volume of 1.0 mL = 1:2 dilution.

The dilutions in the series of tubes are as follows:

Tube #1, 1:5; tube #2, 1:10; tube #3, 1:20; tube #4, 1:40; tube #5, 1:80; tube #6, 1:160; tube #7, 1:320; tube #8, 1:640; tube #9, 1:1280; tube #10, 1:2560

The reciprocal of the dilution in tube #8 (1:640) is 640.

95.

**A.** In chemiluminiscent assays, light is the end product. These assays require special instruments to measure the light emitted in the reaction. Chemicals used to generate light include luminol and luciferase.

96.

**C.** The presence of HBsAg indicates viral replication and that the patient is infectious; this marker can be seen in both acute and chronic infections. The lack of anti-HBs indicates that the patient is not immune to the infection. The presence of anti-HBc and HBeAg with HBsAg indicates a chronic infection.

97.

**B.** The symptoms of fever of unknown origin, lymphocytosis, and lymphadenopathy suggest EBV or CMV infection and lymphoma or leukemia. Heterophile antibodies become positive later than antibodies to viral core antigen (VCA) of EBV. In addition, only about 50% of children less than 12 years of age form heterophile antibodies following EBV infection. The IgM titer of 80 for EBV is consistent with acute EBV infection.

98.

**D.** The case history is typical of a child with X-linked agammaglobulinemia. He presented with chronic and recurrent infections beginning at 5 months of age, when transplacentally acquired IgG had declined. Normal IgG serum level is about 800–1200 mg/dL. The infant had normal T cell function, which rules out combined immunodeficiency and DiGeorge syndrome. “Iatrogenic immunodeficiency” refers to an immunodeficiency following therapy prescribed by a physician.

**99.**

**B.** When a standard screening test for human immunodeficiency virus infection, such as an ELISA, is positive, it is recommended that the sample be repeated in duplicate. If one or both of the repeated tests is reactive, the sample is considered to be repeatedly reactive and needs to be confirmed by a confirmatory test (e.g., Western blot or immunofluorescent antibody). If this test is positive, the sample can be reported as positive. If the confirmatory test is negative, an additional confirmatory test should be performed if the patient has risk factors for HIV infection. If the repeated ELISA tests are both negative, the sample is reported as negative.

**100.**

**A.** The presence of arthritis is suggestive of a number of autoimmune diseases. Protein, RBCs, and casts in the urine are indicative of kidney inflammation. These signs and symptoms along with the rash on the face are characteristic of systemic lupus erythematosus (SLE). A commonly used sensitive screening test for SLE is the antinuclear antibody (ANA) test. The ANA, however, is not specific for SLE. If the ANA were positive, additional autoantibody tests specific for SLE (e.g., anti-Smith) should be performed.

## REFERENCES

- Male, D., Brostoff, J., Roth, D. B., and Roitt, I. (2006). *Immunology*, 7th ed. London: Mosby.
- Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L., and Pfaller, M. A. (2007). *Manual of Clinical Microbiology*, 9th ed. Washington, DC: ASM.
- Murray, P. R., Rosenthal, K. S., and Pfaller, M. A. (2005). *Medical Microbiology*, 5th ed. St. Louis: Mosby.
- Sharon, J. (2002). *Essentials of Immunology & Serology*. Albany, NY: Delmar-Thomson Learning.
- Turgeon, M. L. (2009). *Immunology and Serology in Laboratory Medicine*, 4th ed. St. Louis: Mosby.

